



Natural Enemies

An Introduction to
Biological Control

Ann Hajek

CAMBRIDGE

CAMBRIDGE

www.cambridge.org/9780521652957

This page intentionally left blank

Natural Enemies

An Introduction to Biological Control

Natural Enemies: An Introduction to Biological Control gives a thorough grounding in the biological control of arthropods, vertebrates, weeds, and plant pathogens through use of natural enemies. The book is intended for undergraduate students and others wishing to learn the basics of biological control. Ann Hajek discusses the reasons why biological control is used, and describes different use strategies and associated safety issues, as well as how best to integrate biological control with other types of pest management. She goes on to describe the basic biology of the different types of natural enemies, and gives examples of successful biological control programs. Throughout this book the ecological relationships that make control possible are emphasized and the major strategies for the use of different types of natural enemies detailed, with discussions of the specific conditions under which each strategy is successful in controlling pests.

ANN E. HAJEK is an associate professor in the Department of Entomology at Cornell University, where she teaches a lower-division course on natural enemies, and a graduate course on invertebrate pathology. She has worked on numerous different types of natural enemies and their use to control pest populations. Her research program centers currently on fungal diseases of insect pests, emphasizing the gypsy moth and the invasive Asian longhorned beetle.

Natural Enemies

An Introduction to Biological Control

Ann E. Hajek

Department of Entomology

Cornell University



CAMBRIDGE
UNIVERSITY PRESS

CAMBRIDGE UNIVERSITY PRESS

Cambridge, New York, Melbourne, Madrid, Cape Town, Singapore, São Paulo

Cambridge University Press

The Edinburgh Building, Cambridge CB2 2RU, UK

Published in the United States of America by Cambridge University Press, New York

www.cambridge.org

Information on this title: www.cambridge.org/9780521652957

© Ann Hajek 2004

This publication is in copyright. Subject to statutory exception and to the provision of relevant collective licensing agreements, no reproduction of any part may take place without the written permission of Cambridge University Press.

First published in print format 2004

ISBN-13 978-0-511-18621-9 eBook (EBL)

ISBN-10 0-511-18621-5 eBook (EBL)

ISBN-13 978-0-521-65295-7 hardback

ISBN-10 0-521-65295-2 hardback

ISBN-13 978-0-521-65385-5 paperback

ISBN-10 0-521-65385-1 paperback

Cambridge University Press has no responsibility for the persistence or accuracy of URLs for external or third-party internet websites referred to in this publication, and does not guarantee that any content on such websites is, or will remain, accurate or appropriate.

To Lisa, Jonathan, and Jim

Contents

Preface	page xi
Acknowledgments	xiv

Introduction

Chapter 1	Why use natural enemies?	3
1.1	Historical perspective on chemical pest control	4
1.2	Why consider biological alternatives?	5
1.3	A pest or not?	15
	Further reading	17

Chapter 2	Introduction to biological control	19
2.1	Defining biological control	19
2.2	Natural control	21
2.3	Diversity in biological control	22
2.4	History of biological control	25
2.5	Studying biological control	30
	Further reading	35
	Selected general references on biological control	35

PART I Strategies for using natural enemies

Chapter 3	Classical biological control	39
3.1	Uses of classical biological control	43
3.2	Success in classical biological control	48
3.3	Economics of classical biological control	56
3.4	Methods for practicing classical biological control	56
	Further reading	61

Chapter 4	Augmentation: inundative and inoculative biological control	62
4.1	Inundative biological control	62
4.2	Inoculative biological control	63
4.3	Inundative versus inoculative strategies	65
4.4	Production of natural enemies by industry	68
4.5	Products for use	69
4.6	Regulation	77
4.7	Natural enemies commercially available for augmentative releases	77
	Further reading	79

Chapter 5	Conservation and enhancement of natural enemies	80
5.1	Conserving natural enemies: reducing effects of pesticides on natural enemies	81
5.2	Enhancing natural enemy populations	83
	Further reading	96
PART II	Biological control of invertebrate and vertebrate pests	
	Invertebrates	97
	Vertebrates	98
Chapter 6	Ecological basis for use of predators, parasitoids, and pathogens	101
6.1	Types of invertebrate pests	101
6.2	Types of natural enemies	102
6.3	Interactions between natural enemies and hosts	105
6.4	Population regulation	106
6.5	Is stability necessary for coexistence of natural enemies and hosts?	114
6.6	Microbial natural enemies attacking invertebrates	118
6.7	Food webs	120
	Further reading	123
Chapter 7	Predators	124
7.1	Vertebrate Predators	124
7.2	Invertebrate Predators	126
7.3	Specialist versus generalist predators	138
7.4	Use of invertebrate predators for pest control	140
	Further reading	143
Chapter 8	Insect parasitoids: attack by aliens	145
8.1	Taxonomic diversity in parasitoids	146
8.2	Diversity in parasitoid life histories	149
8.3	Locating and parasitizing a host	157
8.4	The battle between parasitoid and host	160
8.5	Use of parasitoids in biological control	164
	Further reading	168
Chapter 9	Parasitic nematodes	170
9.1	Steinernematidae and Heterorhabditidae	171
9.2	Mermithidae	174

9.3 Use for control	175
Further reading	179
Chapter 10 Bacterial pathogens of invertebrates	180
10.1 Use for pest control	181
Further reading	189
Chapter 11 Viral pathogens	190
11.1 General biology of viruses	191
11.2 Invertebrate viral pathogens	191
11.3 Vertebrate viral pathogens	199
Further reading	202
Chapter 12 Fungi and microsporidia	203
12.1 Fungal pathogens of invertebrates	203
12.2 Microsporidia	211
Further reading	213
PART III Biological control of weeds	
Chapter 13 Biology and ecology of agents used for biological control of weeds	217
13.1 Types of agents	217
13.2 Weed characteristics	218
13.3 Types of injury to plants	219
13.4 Regulation of weed density by herbivores	224
13.5 Measuring impact of biological control	231
Further reading	232
Chapter 14 Phytophagous invertebrates and vertebrates	233
14.1 Invertebrates	233
14.2 Successful attributes of invertebrate herbivores	236
14.3 Strategies for use of phytophagous invertebrates	238
14.4 Vertebrates	248
Further reading	249
Chapter 15 Plant pathogens for controlling weeds	251
15.1 Inundative biological control	251
15.2 Inoculative biological control	254
15.3 Classical biological control	255
Further reading	257

PART IV	Biological control of plant pathogens and plant parasitic nematodes	
Chapter 16	Biology and ecology of antagonists	261
16.1	Types of plant pathogens and their antagonists	261
16.2	Comparing macroecology with microecology	262
16.3	Ecology of plant pathogens and their antagonists	264
16.4	Interactions among microorganisms	266
16.5	Indirect effects	273
	Further reading	276
Chapter 17	Microbial antagonists combating plant pathogens and plant parasitic nematodes	277
17.1	Finding antagonists	277
17.2	Types of antagonists	279
17.3	Strategies for using antagonists to control plant pathogens	281
17.4	Conservation/environmental manipulation	289
17.5	Biological control of plant parasitic nematodes	292
	Further reading	293
PART V	Biological control: concerns, changes, and challenges	
Chapter 18	Safe biological control	297
18.1	Importance of non-target effects	298
18.2	Reasons non-target effects have occurred	301
18.3	Direct versus indirect effects	308
18.4	Predicting non-target effects	309
18.5	Preventing non-target effects	315
	Further reading	317
Chapter 19	Present uses of biological control	318
19.1	Using natural enemies alone?	318
19.2	Control through managing pests	319
19.3	Adding an ecological emphasis to pest management	328
19.4	Biological control in use today	330
	Further reading	336
	Glossary	338
	References	347
	Index	366

Preface

My intent in writing this book has been to present an introduction to the many different types of natural enemies used for biological control, in the context of an overview of biological control methods and uses. This book grew from a course that I've taught for undergraduates at Cornell University. Many of my students have little background in entomology, plant pathology, or weed science but they are interested in biological control. My major goal with my course has been to make it possible for a greater breadth of people to learn about this subject. The first major book focusing specifically on biological control of insect pests and weeds was published in 1964 (DeBach, 1964a) and the first major book focusing on biological control of plant pathogens was published in 1974 (Baker & Cook). Since these first treatises, there have been numerous excellent books on biological control. The majority of these books are more detailed and are intended for professionals in this field. Some introductory books currently available are very basic or only cover a specific type of biological control. Therefore, I saw the need for a book such as this.

I find the interactions between natural enemies and their hosts fascinating and I feel that it makes sense to use these relationships to control pests whenever possible. Our challenge with biological control is to figure out how to manipulate these relationships to control pests. In fact, many ecologists and biological control practitioners are of a mind that a deep understanding of the natural history of pest insects and their natural enemies is one of the most important factors necessary for creating successful biological control programs. Therefore, this book emphasizes the diversity in biology and ecology of natural enemies and antagonists used in biological control.

This book is intended as a basic presentation and readers should not need an extensive background in entomology, plant pathology, or weed science. I have attempted to use scientific jargon as little as possible and have provided a glossary to help with specific terms used in the text. Many of the pests and natural enemies that must be mentioned in a general book on biological control do not have common names as well as their Latin genus and species names, so I've used Latin names when necessary. The common names of major groups are also used, when possible.

The structure of the book is self-evident. First, the necessity for alternatives to synthetic chemical pesticides, the most commonly used type of pest control, is discussed. Then, I go on to define biological control. Of course, there are many different definitions for biological control but I hope that books such as this one will help to create a more unified definition. Biological control is composed of very different use strategies, classical biological control, augmentation, and conservation, and these are presented next. Following are sections

about uses of the different types of natural enemies for control of pestiferous animals, weeds, and plant pathogens. Within each of these sections, the basis for ecological interactions yielding control are presented and then the biology of each type of natural enemy is presented with a description of its use for control. In particular, within the section on control of animal pests, readers might not understand why the pathogens used for control of invertebrates and vertebrates are covered in separate chapters. I feel that these different groups of pathogens (viruses, bacteria, fungi, and microsporidia) are quite well known and have such different biologies that separate treatments are needed. In compensation, the chapters covering these groups are significantly shorter than the chapters on parasitoids and predators. For biological control of weeds, herbivores and plant pathogens are covered separately. For biological control of plant pathogens, antagonists are discussed as a group because in many cases, the biological control agents are not easily segregated by mode of action since several modes of action are frequently utilized by one species of antagonist. Toward the end of the book, the controversial subject of the safety of biological control is discussed followed by present uses of natural enemies for control, including their integration with other control tactics.

The field of biological control is rapidly growing. There is a wealth of information on a diversity of types of biological control. In some cases, biological controls are operational for pest control. There are many systems, however, where researchers see the potential for use of certain organisms for control but research and development are still under way and the system has not yet been effectively put to use. In this book I have tried to emphasize examples of biological control that are in use, while describing some pest/natural enemy systems that are close to utilization and only occasionally discussing systems that are simply tantalizing. Those readers interested in biological control agents that hold promise but are not yet being used are referred to the further reading suggestions at the end of each chapter as well as the large number of reviews and enormous number of primary papers in the scientific literature. Because this is intended as a textbook or general resource I have not provided exhaustive citations throughout the text, as would be found in edited volumes or refereed journal articles. Within each chapter, citations are provided sparingly and usually only when very specific information is presented. At the end of each chapter, a list of the most pertinent and recent summary readings along that subject is given for those who might want to read further.

Generalization pertaining to biology must always be followed by exceptions. In fact, making generalizations virtually means leaving out at least some of the fascinating variability found in biological systems. There are many tales of amazing interactions and relationships among natural enemies and their hosts or prey and only a small fraction of these could be included in this book. The diversity of manipulations of biological systems for pest control also made it difficult

to decide which examples to include in a book such as this. My emphasis has been on providing a glimpse of the diversity of natural enemies and biological control approaches. In summary, with this book I hope that I have shared my excitement about the field of biological control and that you have also become fascinated with the practice and potential of using natural enemies to control pests.

Acknowledgments

This book would not have been possible without the help of many kind and helpful colleagues. I want to thank Gary Blissard, Bernd Blosssey, Bill Bruckart, Jørgen Eilenberg, Curtis Ewing, Roger Fuester, Molly Hunter, Albrecht Koppenhöfer, Lerry Lacey, Sandy Liebhold, John Losey, Eric Nelson, Jan Nyrop, Bob Pemberton, and John Vandenberg for reviewing drafts of specific chapters. Greg English-Loeb, Carol Glenister, and Joyce Loper did more than their share and need special thanks. Many scientists helped by answering my endless questions, providing unpublished data, and reviewing specific sections of text, including Michelle Cram, David Greathead, Micky Eubanks, Kathie Hodge, Rick Hoebeke, Richard Humber, Linda Lear, Chris Lomer, Clay McCoy, Don Rutz, Joop van Lenteren, Graham White, and many others. I also want to thank Art Bloom, Harry Green, George Hudler, and Peter Price for sharing with me their expertise on how to write a book (and try to maintain one's sanity).

Alison Burke graciously provided her artistic expertise in assisting with figures for this book. This book would not have been so lovely without her devotion and excellent artistry. I thank Kent Loeffler for helping me in the early stages with figures for this book. I also want to extend my thanks to the many scientists who provided their illustrations and photos for this book.

There are many scientists to thank for teaching me about biological control. First, Don Dahlsten, Ken Hagen, Leo Caltagirone, Junji Hamai, and Robert van den Bosch were instrumental in sharing their expertise with predators and parasitoids while I studied at the University of California, Berkeley, Division of Biological Control. After I moved to the Cornell campus, Dick Soper, Don Roberts, and Bob Granados shared their enthusiasm and knowledge about insect pathogens. Cornell has been an exciting location for working on biological control and I have learned much from my many colleagues working on a great diversity of types of biological control and especially the fungal pathogen gang, Rich Humber, John Vandenberg, and Steve Wraight.

The folks working with me while I was writing this book, Mike Wheeler, Alison Burke, Monica Bertoia, Italo Delalibera, Thomas Dubois, Melanie Filotas, Josh Hannam, Mike Jackmin, Jim McNeil, Victoria Miranda, and Charlotte Nielsen, have been extremely helpful with many aspects of putting this book together as well as with their patience when I was busy with this book.

I want to thank my editor, Tracey Sanderson, for her steadfast faith from the beginning that this book could and would be written. I sincerely thank the Danish National Bank and Jørgen Eilenberg for their gracious support while I began writing this book while on sabbatical

in Copenhagen. Jørgen and Chris Lomer provided early inspiration when we worked together in Copenhagen.

My daughter, Lisa, has been integrally involved in helping with various aspects of this book while my son, Jonathan, provided hugs. Most of all, my husband Jim provided support throughout, especially when this project seemed never-ending.

Introduction

Why use natural enemies?

Humans share the planet earth with some 10 million species of organisms. Each species eats, grows and reproduces in different ways in different locations around the world but virtually no species does this in isolation. All species are interconnected to some extent, with some organisms more dependent on others, especially those higher in the food chain. Tigers would not live long without their prey being available, just as rabbits would not survive for long without plants to eat. Humans have quite a dominant position in many ecosystems and they depend on many other species for food and shelter. Especially because the influence of humans is so pervasive throughout the world, humans also compete with many organisms and we generally think of many of these competitors as “pests.”

Man has been plagued by “pests” since time began. A pest can be formally defined as any organism that reduces the availability, quality, or value of some human resource (Flint & van den Bosch, 1981). The definition of pest needs to be broad due to the great diversity in the ways that pests affect humans. The resources in question can be a plant or animal grown for food, fiber or pleasure (e.g., pets, plants in recreation areas). Another resource is human health and well-being, making organisms directly affecting human health, such as mosquitoes, pests too. Pests are as diverse taxonomically, ranging from microorganisms to mammals, as they are in the ways that they compete with humans. With such variability comes a variety of adaptations, and some organisms competing with humans are tough adversaries.

There are many different means for controlling pests but this book is concerned only with methods using living organisms to control pests, a strategy called biological control. We will therefore not be covering all pests but only those specifically targeted by biological control. The major types of pests that are addressed by biological control include weedy plants, microorganisms attacking plants (often crop plants or forest trees), invertebrates (especially arthropods that often attack plants or animals), and vertebrates.

I.1 Historical perspective on chemical pest control

Humans have always needed to control pests affecting them directly, such as mosquitoes or bed bugs, or competing with them for a great diversity of resources. Through the ages pest control practices have changed dramatically. The earliest known record for the use of naturally occurring compounds for pest control was in ≈ 1000 BC, when the Greek Homer mentioned using sulfur as a fumigant. In the 1800s, tobacco extracts and nicotine smoke were applied for insect control. In 1867, we see the first mention of a mixture concocted for pest control that became widely used; Paris green, an arsenic-based compound, was developed and applied against Colorado potato beetle in the USA. Bordeaux mix, a combination of copper sulphate and hydrated lime, was developed in 1882 in Bordeaux, France, for control of plant pathogenic fungi on grapes and other fruits.

Throughout these times, the overriding methods for pest control were cultural controls, such as leaving fields to lie fallow and rotating crops. For example, when soybean crops are rotated with corn, the soil-dwelling nematodes that attack soybean roots are nearly eliminated so that soybeans can again be planted. Other cultural controls included practices such as altering dates for planting and harvesting, using trap crops, planting mixtures of crops, managing drainage and removing crop residues that harbor pests. Growers were basically manipulating and augmenting the naturally occurring processes of pest suppression.

Between World Wars I and II, several developments took place, setting the stage for major changes in pest control. Industries developed methods for large-scale production and chemists vastly improved their abilities to synthesize chemicals. In 1939, both DDT for control of insects and 2,4-D for control of weeds came on the scene. These extremely effective compounds revolutionized pest control. Since that time, a cascade of different compounds, belonging to an increasing number of chemical classes, have been synthesized for pest control. Most of the early compounds were effective against a broad spectrum of pests, killed pests very quickly, and were relatively easy to apply using spray equipment. Availability of these synthetic chemical pesticides changed the potential for successful harvests and, consequently, use of these compounds skyrocketed.

Use of pesticides over time increased but these changes are not easy to quantify. Figure 1.1 illustrates the increase in value of different types of pesticides on the worldwide market from 1980 to 1999. While the majority of this pesticide use occurs in North America and Europe (56%), use in Asia and South America is also significant (Bateman, 2000). Between 1980 and 2000, the total value of pesticide sales increased approximately 2.5 times. Looking at the weight of pesticides applied can be a misleading statistic because over time, the potency of pesticides has increased, confounding comparisons through

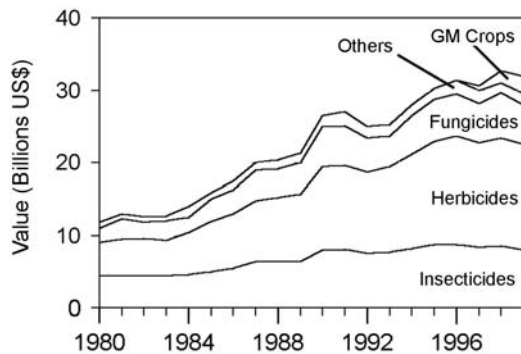


Fig. 1.1 Worldwide pesticide markets in the final two decades of the twentieth century. Data compiled from the annual reviews of the British Agrochemicals Association. (Bateman, 2000.) GM crops, genetically modified crops.

time. Data for the amount of land on which pesticides are applied are rarely available. A major fact to be gleaned from Fig. 1.1 is that among the numerous types of pesticides, the use of herbicides increased substantially from 1980 to 1999. Surprisingly, although genetically modified crops began to be used, they are not used very extensively in contrast to the publicity they have received. The bottom line is that as of 1990, an estimated 2.5 million tons of pesticides were applied each year worldwide at a cost of \$20 billion. In the USA alone, 500,000 tons were used yearly at a cost of \$4.1 billion. Today, synthetic chemical pesticides are clearly the most commonly used method for pest control (OTA, US Congress, 1995).

1.2 Why consider biological alternatives?

Synthetic chemical pesticides are used so widely because they often work very well for controlling pests. However, pesticides are not always the correct answer; sometimes they cannot control pests effectively for a variety of reasons. The major reasons that alternatives to synthetic chemical pesticides have been developed are presented below. In describing these scenarios, control of arthropods (i.e., insects and mites) will be used as examples, although similar issues occur relative to control of weeds and plant pathogens.

1.2.1 The pesticide treadmill

Although synthetic chemical pesticides are still the pest control method most widely used by many people, we are finding that there are growing reasons to consider alternatives. When pesticides are applied to control arthropods, naturally occurring controls are frequently severely disrupted and natural enemies normally living by consuming a pest are no longer abundant, or even present. Therefore, when the target pest reinvades the area, there are no natural enemies present and the target pest population increases again, frequently to higher densities than were present initially (= target pest resurgence) (Fig. 1.2). Figure 1.3 shows the growth of an outbreak in

Fig. 1.2 Target pest resurgence can occur when natural enemies are destroyed. Pesticides often kill a higher proportion of natural enemies than pests so that after application, the pest can increase again rapidly. (From Flint & Dreistadt, 1998.)

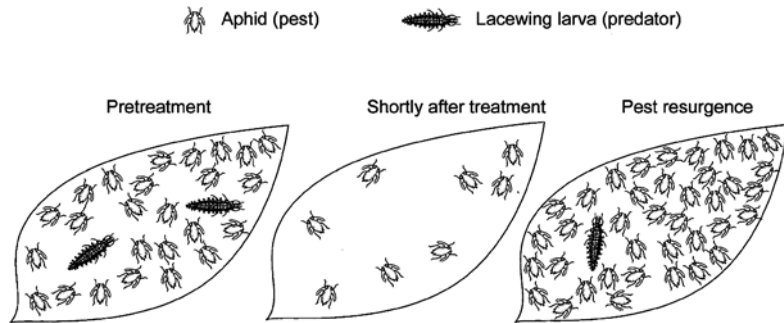
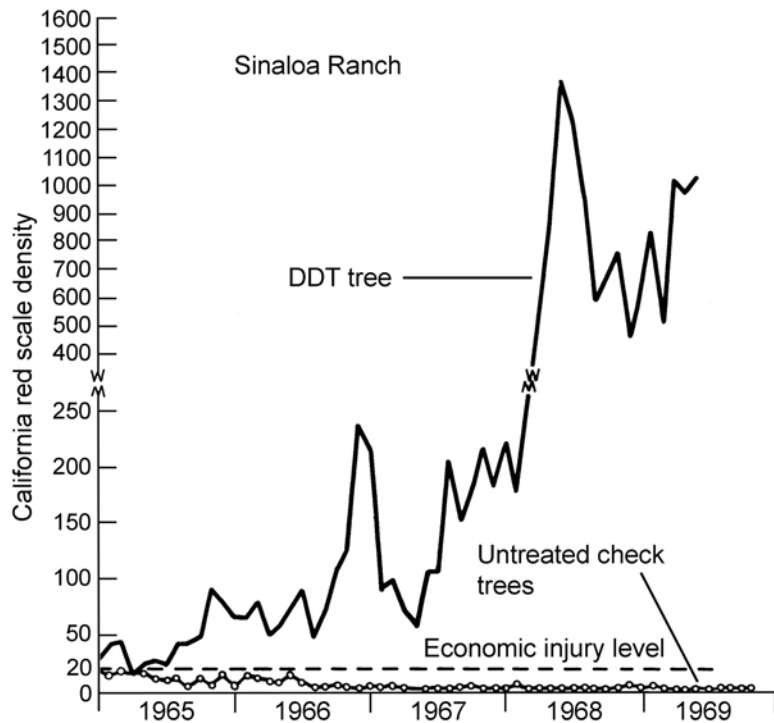
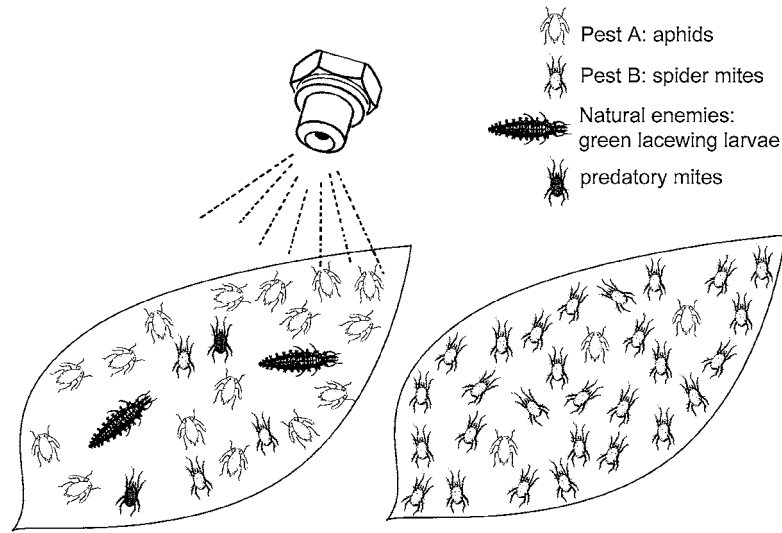


Fig. 1.3 Increases in California red scale, *Aonidiella aurantii*, on citrus tree associated with light monthly sprays of DDT, compared with nearby untreated trees under biological control. (From DeBach et al., 1971.)



a target pest, the California red scale, due to light, regular spraying of DDT.

Since all of the natural enemies are often killed when pesticides are applied, other insects that had not previously been pests can increase to densities that cause damage, because the natural controls previously maintaining their populations at low densities are no longer present or abundant enough for control (Fig. 1.4). This scenario of a secondary pest outbreak can be demonstrated with the increase in the European spruce sawfly, which was under biological control until DDT was applied to control spruce budworm, *Choristoneura fumiferana*, in the same forest (Fig. 1.5). New York State apples provide an example of the diversity of secondary pests that can become



A pesticide applied to control pest A also kills natural enemies that are controlling pest B.

Released from the control exerted by natural enemies, pest B builds up to economically damaging levels.

Fig. 1.4 Secondary pest outbreaks occur when pesticide applications kill the natural enemies that have been controlling a species that has not been a pest. Without natural control, this species increases and can become a “secondary pest.” For example, a pesticide applied to kill Pest A (aphids) killed aphids and their predators, the green lacewings, but also killed predatory mites, resulting in a secondary pest outbreak of Pest B (spider mites), previously at lower densities due to predatory mites. (From Flint & Dreistadt, 1998.)

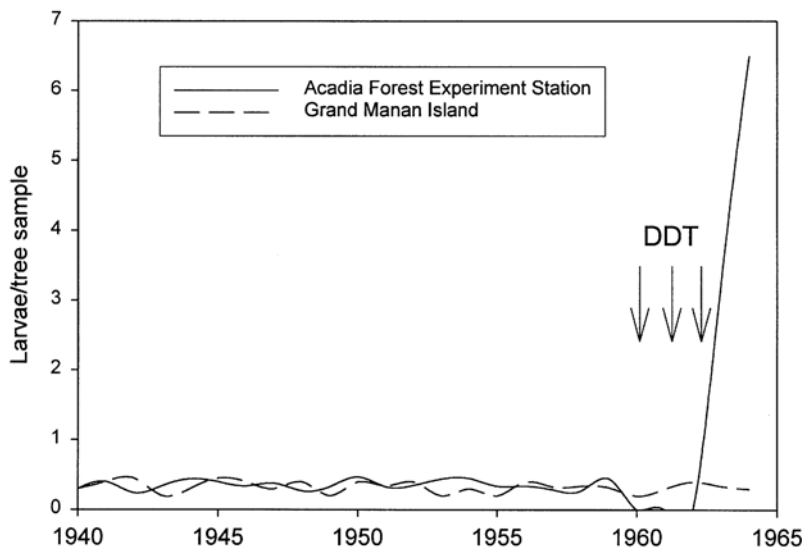


Fig. 1.5 Increases in populations of a secondary pest in New Brunswick, Canada. European spruce sawfly (*Gilpinia hercyniae*) had been under biological control since 1940 but from 1960 to 1962 DDT was sprayed to control a different pest, spruce budworm. Spruce sawfly populations plummeted and their parasitoids could no longer be found, subsequently leading to an outbreak of spruce sawfly in 1964. On Grand Manan Island, no DDT was applied and an outbreak did not occur (Neilson & Elgee, 1965.)

problematic due to the application of broad-spectrum insecticides for control of different primary pests (Table 1.1). In this case, several different insect and mite species, previously not pests, can increase to pest levels due to severe reductions in the populations of their natural enemies, thus demonstrating that a diversity of problems can arise due to outbreaks of secondary pests.

A third effect of extensive use of pesticides can be development of pesticide resistance (Fig. 1.6). Resistance can develop when a pesticide is extremely effective and the majority of the pest population dies after an application. However, sometimes a few individuals remain

Table 1.1 Key and secondary arthropod pests in apples in New York State		
Type of pest	Species	Type of damage
Key pests	Codling moth (<i>Cydia pomonella</i>)	For all key pests, larvae bore into developing apples
	Plum curculio (<i>Conotrachelus nenuphar</i>)	
	Apple maggot (<i>Rhagoletis pomonella</i>)	
	European apple sawfly (<i>Holocampa testudinea</i>)	
Secondary pests	Spotted tentiform leafminer (<i>Phyllonorycter blancardella</i>)	For all secondary pests, apples are not directly damaged but overall tree health can be impacted
	European red mite <i>Panonychus ulmi</i>	
	White apple leafhopper (<i>Typhlocyba pomaria</i>)	
	Apple/Spirea aphids <i>Aphis pomi</i> and <i>A. spiraecola</i>	
	Twospotted spider mite (<i>Tetranychus urticae</i>)	

(A. Agnello, personal communication).

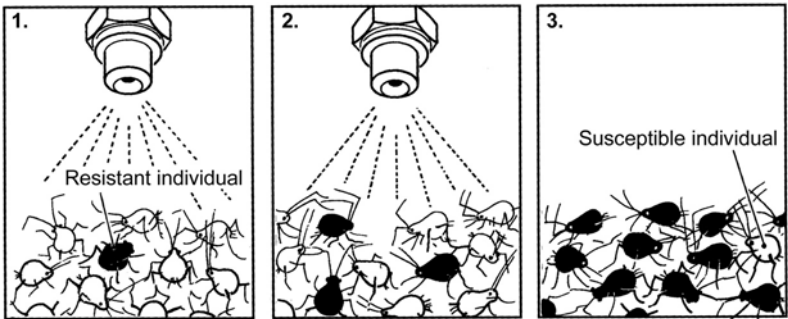


Fig. 1.6 Pest populations can develop resistance to pesticides through natural selection. 1. When pesticides are applied, most individuals are killed but a few are less susceptible and these remain. 2. The less susceptible individuals or their progeny are less likely to die with subsequent applications. 3. After repeated applications, the resistant or less susceptible individuals predominate and applying the same pesticide is no longer effective. (Flint & Dreistadt, 1998.)

that are physiologically different and can tolerate the pesticide. The “new” strain of the pest that has been created is resistant to the pesticide and the population can then increase even when the pesticide is reapplied. Overusing the pesticide in response to lack of control only hastens the occurrence of resistance throughout the pest population. Eventually, the pesticide applied has no effect on the pest and a different control strategy must be used. It is often assumed that when a new material is developed, it will only be a matter of a few years

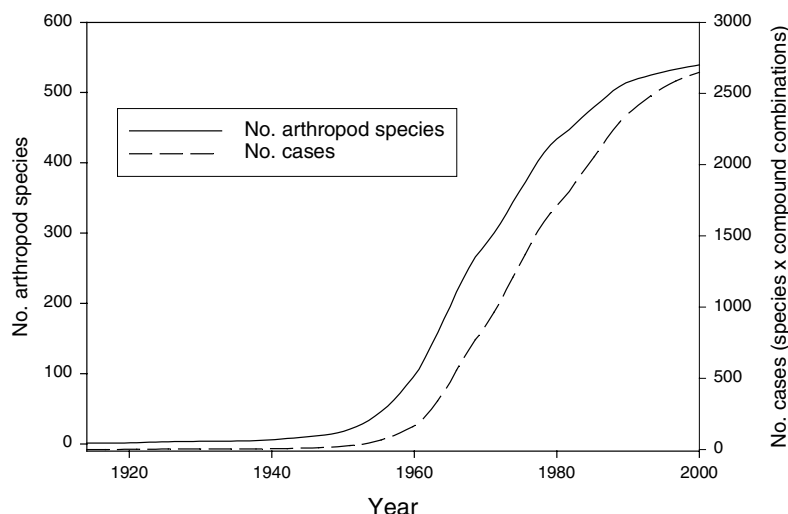


Fig. 1.7 Numbers of arthropod species resistant to pesticides and the total of resistant species \times compound combinations (= cases) in the United States from 1914 to 2000. (Redrawn from Mota-Sanchez *et al.*, 2002.)

before resistance to the new compound begins to develop in some pest population.

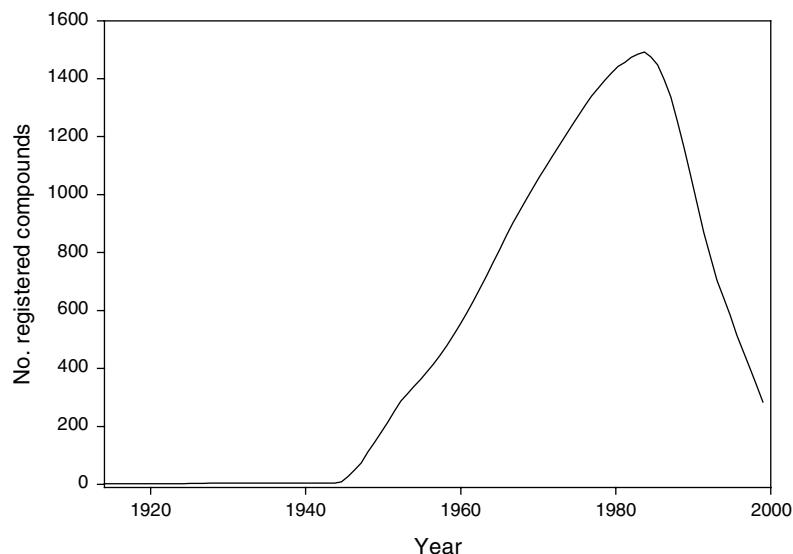
In fact, resistance to DDT was first seen in 1946 in houseflies, only 7 years after DDT began being used. By 1948, pesticide resistance was seen in 14 species and by 1990 over 500 species of arthropods displayed resistance to insecticides (Fig. 1.7). First, when resistance to insecticides begins to develop, growers characteristically apply more insecticide, often not realizing that the lack of control is due to resistance. Next, growers might switch to a closely related pesticide, but once pests develop resistance to one pesticide in a pesticide class, they are often at least partially resistant to other similar pesticides. The grower also might choose another class of pesticides, for example switching from organophosphate insecticides to pyrethroids, under the assumption that the pest had acquired at least partial resistance to all organophosphates. However, pests can be resistant to several classes of pesticides at the same time and resistance can eventually develop to this second choice of control agent. As a double whammy, frequently the alternative pesticide can be more costly. For example, with development of resistance to DDT, malathion was substituted at five times the cost but when resistance developed to malathion, fenitrothion, propoxur, or deltamethrin were often substituted by growers at 15–20 times the cost.

These three phenomena together (target pest resurgence, secondary pest outbreaks, and development of resistance in pest populations) have been termed the pesticide treadmill. They lead to increasing dependence on pesticides, seemingly an addiction for use of this type of control.

1.2.2 Fewer pesticides are available

Due to the development of resistance to classes of pesticides, there is a constant demand for new types of pesticides. However, the costs of developing and registering new pesticides have increased over time.

Fig. 1.8 Numbers of registered pesticides for arthropod control in the USA from 1914 to 1999. (Redrawn from Mota-Sanchez *et al.*, 2002.)



Since 1970, there has been a significant slow down in the rate of new pesticides being introduced to the market. In addition, due to increased regulation, some of the pesticides that have been available for many years are no longer legally available for application. For both of these reasons, in many countries there are fewer pesticides registered and thus available for use (Fig. 1.8). As one example, a mainstay for control of soil-borne pathogens and pests as well as storage diseases of fruits and vegetables has been fumigation with methyl bromide. In the year 2010, this chemical will be banned worldwide due to its role in ozone depletion (Ristaino & Thomas, 1997) so alternative controls must be used. In summary, there is a trend toward fewer synthetic chemical pesticide options due to increased resistance to existing insecticides, banning some compounds, and decreased development and registration of new compounds.

1.2.3 Synthetic chemical pesticides aren't always the answer

There are some situations in which chemical pesticides are not the most appropriate choice for controlling pests. One example would be introduced exotic organisms that become pests; it has been estimated that 30,000 exotic organisms have been introduced to the USA. In fact, invasive species are now considered a major problem worldwide due to the increasing human population frequently moving organisms around the globe and thereby altering ecosystems at an increasing rate. Many invaders become pestiferous largely due to the fact that they are no longer associated with the natural enemies with which they coevolved. Among pests in agriculture, approximately 20–40% have been introduced from elsewhere. Most are accidental introductions, although a small percentage of these were purposeful introductions such as crop plants and honeybees. Some were purposeful introductions with unexpected side effects. For example, the weed kudzu was introduced to the southeastern USA to control erosion

and has since spread rampantly through most of the southeast, becoming a problematic weed. Introduced organisms are not always identified quickly, so they establish and become ubiquitous before it is possible to eradicate them. It is difficult to imagine how a synthetic chemical pesticide can easily solve such a problem as a fast-growing weed, without continual human intervention and its associated costs. Problems due to such pests are therefore often not readily addressed using synthetic chemical pesticides because more permanent control is what is needed. Classical biological control has frequently been successfully used against such pests (permanently introducing natural enemies from the land of origin of the pest). Unfortunately, by all predictions, accidental introductions of invasive species will only continue with the increased global movement of humans and materials (see below).

Synthetic chemical pesticides, for a variety of reasons, simply cannot control some pests. Damaging stages of numerous arthropod pests live in the soil, especially those that feed on roots. Control of soil-dwelling arthropods is not as straightforward as control of externally feeding arthropods. It can be very difficult, if not impossible, to apply pesticides that will reach soil-dwelling arthropods and plant pathogenic nematodes. In the past, soil was sometimes fumigated due to this difficulty but now many fumigants, and two of the most effective nematicides, can no longer be applied in the USA. To add to this problem, the fumigants that are now available are very costly.

No chemicals are capable of controlling some pests. Just as there is presently no control for the common human cold that is caused by viruses, there are no chemical options for control of viral diseases of plants. Therefore, general control tactics employed for control of plant pathogenic viruses include cultural practices such as rotating crops and altering planting dates, use of insecticides to control insects vectoring the viruses, and use of virus-resistant strains of plants.

Sometimes a crop or habitat is just not amenable to use of synthetic chemical pesticides. For rangeland weeds and insects, the areas impacted by these pests can be huge. However, rangeland is not high-value land and the yield from the land often cannot support the cost of spraying such huge acreages. For vegetable crops, problems can be due more to the small acreages planted with a diversity of crops. Pesticide manufacturers have to develop and register individual pesticides for particular crops. Pesticide producers have little monetary incentive to develop and register pesticides that will be used on such small areas. Therefore, there are often not many chemical options for control of pests in many crops planted on smaller acreages. As a third case where chemical pesticides are not optimal, aquatic weeds can be immense problems when they block waterways. These are usually controlled by manual removal and by applying herbicides, but such solutions are only temporary and the problem then usually recurs. Because repeatedly applying herbicides to water can lead to presence

Box 1.1 | Rachel Carson

Rachel Carson was a quiet person who loved nature and whose writings had a profound influence on the creation of the level of environmental consciousness present today. She was born in 1907 in rural Pennsylvania, far from the ocean but, as her career developed, she followed her fascination with marine biology. She spent time at Wood's Hole Biological Laboratory on Cape Cod, Massachusetts, and her training was completed with a master's degree in zoology from Johns Hopkins University in 1932. In 1936, she began working as a scientist and editor for the US Fish and Wildlife Service, where she continued working for 15 years. She never married, caring for her mother and adopting her grand nephew after his parents died. During this time, she began writing about the natural history of the sea for the public. In 1952, the same year that she completed her prize-winning book *The Sea Around Us*, she resigned from her position as Editor-in-Chief of the US Fish and Wildlife Service to be able to concentrate on her writing.



Rachel Carson. Photo by Brooks Studios, courtesy of the Lear/Carson Collection.

With some reluctance, after World War II Rachel turned her focus from the sea to the land. She was an avid birder and she was very aware of bird deaths linked with pesticide spraying. As she investigated further, she became disturbed by the misuse of synthetic chemical pesticides. She decided to take on the responsibility

of informing the public about the side-effects of pesticide use by writing a book. Originally, she planned on using the title “Silent Spring” for a chapter on effects of pesticides on birds but eventually, in 1962, this title was used for the entire book.

By 1960, Rachel was already fighting breast cancer yet she persevered with publication of her book although she knew that unpleasantness would certainly follow publication of *Silent Spring*. As she had expected, the chemical industry and some members of the US government vehemently charged that she was an alarmist. Yet, Carson’s message was unwavering: she proposed stopping the uncontrolled use of synthetic chemical insecticides that had long-lived activity. She demanded creation of new policies to protect humans and the environment. Her quarrel was with misuse of this technology for which the long-term effects were not known and she insisted on the fundamental rights of individuals to be free from contamination with toxic chemicals without their consent. Her book became a best seller and she lived long enough to see the issues she had raised discussed on television, in the US Congress, and in the British House of Lords. Many credit this quiet naturalist and excellent author with providing the sparks that initiated movements to protect the environment. Certainly, the increase in interest in biological control that began in the 1970s was spurred by desires to find alternative pest controls causing minimal impact to the environment.

of herbicides throughout the environment, including in drinking water, such a control option is often avoided if possible.

1.2.4 Human health and environmental concerns

The first general outcry by the public against use of synthetic chemical pesticides was championed by Rachel Carson, who wrote *Silent Spring*, published in 1962 (Box 1.1). Since the development of synthetic chemical pesticides, pesticide use had been out of control and there were few if any regulations regarding use of pesticides. As an example, a Tennessee Game & Fish Commission biologist cited an application of 10% dieldrin granules (a compound more toxic than DDT) at 30 pounds/acre for Japanese beetle (*Popillia japonica*) control in a recreational area. The granules were so thickly applied that they covered picnic tables and parents and children were told to brush them off of tables before eating (Graham, 1970). Excessive applications such as this resulted in extensive mortality of animals higher in the food chain than insects, for example, birds and fish. Rachel, working as a wildlife biologist, became aware of these environmental side-effects. She decided to write a book about this broad scale, unregulated application of toxins and, in the book, urged the government to investigate the effects of pesticide use and regulate pesticide application. President Kennedy read the book and was instrumental in initiating studies of the type Rachel had urged. The book generated extensive controversy and, despite efforts by the chemical industry to suppress it, *Silent Spring* became a best seller. It is generally credited as the trigger that started the environmental movement. In 1970,

President Nixon created the US Environmental Protection Agency as a direct repercussion from this controversy and today, this organization regulates uses of insecticides, herbicides, and fungicides for pest control. In fact, worldwide, the use of pesticides is regulated by the respective governments in many countries.

Rachel Carson was correct that insecticides were having side-effects on animals and the environment. In the USA, in 1991, it was estimated that approximately 3 kg of pesticide per hectare were applied to about 160 million hectares/year. With this level of application, the naturally occurring flora and fauna are certainly exposed to pesticides. There are direct effects on non-target animals and plants, some being lethal. However, some effects are sublethal, affecting health and reproduction while not killing the less-susceptible species. A classic case was the reproductive failure in predatory birds, often attributed to eggshell thinning, caused by DDT (Pimentel *et al.*, 1992). While DDT has been banned in the USA, it is still used in some South American countries, where numerous migratory bird species overwinter. Another environmental effect is the reduction in sperm production associated with a commonly used soil fumigant, DBCP. Recent studies are suggesting that commonly used herbicides are possibly linked with decreasing amphibian populations. There are also effects due to pesticide residues remaining in the soil or being leached into the water. With the levels of pesticides applied in the USA, one can easily imagine that traces of pesticides occur in drinking water in some areas.

We know that pesticides can have effects on humans but different pesticides at different doses have differing effects. There can be acute effects, causing minor symptoms such as skin or eye injuries but, with exposure to high levels, mortality is possible after exposure to some materials. The effects of chronic exposure to lower levels of pesticides (exposures at doses lower than those causing acute effects and usually over a long period) are more difficult to predict.

To their credit, the chemical companies that develop and market synthetic chemical pesticides are now producing compounds that are much safer for humans and the environment, but still effective for pest control. Regulations are in place in developed countries to ensure safer, yet efficient, use. In developed countries, regulations regarding pesticide use are also becoming stricter. For example, in Denmark, Sweden, and the Netherlands, legislation mandated a 50% reduction in use of agricultural pesticides to be effective by the year 2000 (Matteson, 1995). In the USA, legislation has banned a number of chemical pesticides, and supported use of alternative pest control strategies. The Food and Agriculture Organization (FAO) of the United Nations has adopted a code on distribution and use of pesticides that promotes integrated pest management (Chapter 19) and natural pest control strategies.

However, the pesticides that are banned in the USA and Europe are often still being produced and/or sold in developing nations, where

they are applied without regulation or with little enforcement of regulations.

Many pesticides that have been banned or whose use has been severely restricted in industrialized countries are still marketed and used in developing countries. These chemicals pose serious risks to the health of millions of farmers and the environment.

(FAO Director-General Dr. Jacques Diouf; J. Harris, 2000)

It is very difficult to estimate the extent of effects of chemical pesticides on human health in developed countries and more difficult still in developing countries. In 1992, the World Health Organization estimated that 25 million cases of pesticide poisoning and 20,000 unintentional deaths occur each year, mostly among agricultural workers and rural communities (WHO, 1992). One survey from Nicaragua suggested that two-thirds of cases of pesticide poisoning are not reported. A summary stated that “50% of all pesticide related illnesses and 72.5% of recorded fatal pesticide poisonings occur in developing countries, although these countries account for only 25% of the pesticides used world-wide” (J. Harris, 2000). While more than 80% of pesticides are applied in developed countries, 99% of poisonings occur in developing countries, where regulation and education systems are not as well established.

1.3 | A pest or not?

The goal of biological control is to control pests. The status of a species as a pest at one time does not mean the species will always pose problems. The subjectivity of designation as a pest is illustrated by the fact that species that are pests to some people can be considered beneficial by others. A case in point would be the Halloween lady beetle, *Harmonia axyridis*, introduced to the USA to control aphids. Unfortunately, this beetle species often forms large aggregations in sheltered locations to spend the winter. In the northeastern USA, these beetles find their way into houses where they happily take up residence for the winter, most often being considered a nuisance and therefore, a pest. Therefore, these Halloween lady beetles are seen as beneficial biological control agents by some and nuisances by others.

The point has been made that some organisms considered pests requiring control are sometimes not really causing serious damage. As early as c. 1915 in California citrus, even a few tiny red scale insects or discolored spots due to feeding by other insects (cosmetic damage) decreased profits even if the taste or nutritive value of oranges were not affected. As in many crops today, pests in California citrus are controlled to meet cosmetic standards that often require complete eradication of arthropods from a field. Luckmann & Metcalf (1994) provide a more ecologically based view regarding the presence of pests in crops. “Pest-management concepts dictate a tolerant approach to

pest status. Indeed, it may be that not all pests are bad and that not all pest damage is intolerable.”

Why some species become pests and others do not has been of great interest in biological control. When pest species become very abundant, understanding the cause for the perturbation allowing population increase can help toward developing methods for controlling the pest. Pests can be native species whose numbers have increased because new opportunities are offered due to human activity. For example, when crops are planted as monocultures, previously little-known native species that could feed on the crop plants become important pests due in part to the abundance of a suitable host plant. Potatoes are not native to North America but to South America. When they were first planted in North America, a previously poorly known beetle found these plants that it could eat and its populations increased phenomenally. This is the Colorado potato beetle (*Leptinotarsa decemlineata*) and this is a case of a native insect attacking an introduced plant for which it was pre-adapted. Subsequently, the Colorado potato beetle was introduced to Europe, where it maintained its status as a major pest of potatoes. However, in Europe, the Colorado potato beetle is an introduced insect attacking an introduced plant species.

It has been estimated that 60–80% of all pests are native to the areas where they are pests. Yet, many examples of successful biological control involve pests that have been introduced from one area to another. We refer to such organisms as invasive, introduced, or exotic, while species that evolved in that area are native or endemic. Due to recent controversy regarding the increasing importance of invasive species as pests, this group will be discussed in more depth.

1.3.1 Invasive species

Movement of species to new locations around the world has been very common throughout human history. Many crop plants and domesticated animals were first moved by humans so long ago, and have been moved so extensively since then, that it is difficult to trace exactly where the original strains came from. The rate at which species could be moved long distances really only began to increase once ocean-going sailing ships began to be built in the fifteenth century.

Wherever people traveled, they brought with them the plants they knew how to grow and use for food and, eventually, the animals that they knew how to grow or hunt. On oceanic islands, the waves of people following the explorers were often sealers and whalers and they purposefully brought and released goats and rabbits, while rodents and pet animals were released unintentionally. The native flora and fauna on islands is often characterized as having few species, which are not well adapted to competition or predation. Thus, the fragile endemic species on islands have been severely impacted by invasives.

In the mid-1800s, European settlers in Australia and New Zealand who were far from home, and Europeans curious about exotic species

and potential commercial exploitation of new species, formed so-called “acclimatization” societies. The goal of such societies was specifically to foster importation and establishment of exotic species. Such acclimatization societies were an extreme cause for introducing new species, but where such societies did not occur new species were still introduced but just not as purposefully. As global trade and travel increased, so did the number of organisms that were inadvertently moved from geographic area to geographic area. Changes in land use and destruction of natural areas opened niches for invaders to become established. It has been hypothesized that environmental changes such as warming oceans and changes in large-scale disturbance regimes, for example suppressing forest fires, leave natural systems in imbalance so that invaders more easily become established.

In the USA, it has been estimated that there are more than 50,000 species that are exotics while a summary of six countries (the USA, the British Isles, Australia, South Africa, India and Brazil) estimated that 120,000 invasive species have become established (Pimentel, 2002). Estimates from 14 countries suggest that from 7–47% of the species of terrestrial plants present have been introduced and of these, approximately 15% have become pests. Ultimately, there is also a cost due to invasives. Invasive species have been estimated as costing the USA more than 130 billion dollars per year in damage to agriculture, forests, rangelands, and fisheries.

When pests attack a commercial product it is relatively easy to ascribe a cost to their impact but it is much more difficult to ascribe monetary values to species invading our native flora and fauna. In more recent years, interest has grown regarding exotic species that become established and then outcompete species of the native flora and fauna. Such introductions that affect the biodiversity of an area have been referred to as “biological pollution.” Certainly, the effects from this type of invasion are much more difficult to document, because in many cases we have not documented the standard patterns of activity and abundance for the majority of species occurring as part of our flora and fauna prior to the introduction. Without such information about the initial abundances, it is difficult to quantify changes due to invasives.

FURTHER READING

- Carson, R. *Silent Spring*. Boston, MA: Houghton-Mifflin, 1962.
- Denholm, I., Pickett, J. A. & Devonshire, A. L. (eds). *Insecticide Resistance: From Mechanisms to Management*. Wallingford: CABI Publishing, 1999.
- Graham, F. *Since Silent Spring*. Boston, MA: Houghton-Mifflin, 1970.
- National Research Council. *Ecologically Based Pest Management: New Solutions for a New Century*. Washington, DC: National Academy Press, 1996.
- Perkins, J. H. *Insects, Experts, and the Insecticide Crisis: The Quest for New Pest Management Strategies*. New York: Plenum Press, 1982.
- Pimentel, D. (ed.) *Biological Invasions: Economic and Environmental Costs of Alien Plant, Animal, and Microbe Species*. Boca Raton, FL: CRC Press, 2002.

Pimentel, D., Acquay, H., Biltonen, M., Rice, P., Silva, M., Nelson, J., Lipner, V., Giordano, S., Horowitz, A. & D'Amore, M. Environmental and economic costs of pesticide use. *BioScience*, **42** (1992), 750–760.

US Congress, Office of Technology Assessment (OTA). *Biologically Based Technologies for Pest Control*. OTA-ENV-636. US Government Printing Office, 1995.

Chapter 2

Introduction to biological control

The amount of food for each species of course gives the extreme limit to which each can increase; but very frequently it is not the obtaining food, but the serving as prey to other animals, which determines the average numbers of a species.

(Darwin, 1859)

2.1 | Defining biological control

Populations of all living organisms are, to some degree, reduced by the natural actions of their predators, parasites, antagonists, and diseases. This process has been referred to as “natural control,” but when pests are controlled, this is often called biological control (sometimes shortened to biocontrol) and the agents that exert the control are frequently called natural enemies. Humans can exploit biological control in various ways to suppress pest populations. The varied approaches for manipulating the activity of natural enemies to control pests differ in how much effort is required, who is involved, and the suitability of the approach for commercial development.

Biological control has been defined many times but a commonly accepted definition is provided below.

The use of living organisms to suppress the population of a specific pest organism, making it less abundant or less damaging than it would otherwise be

(Eilenberg et al., 2001).

To understand the basis for this definition, we need to discuss why biological control is used. Of course, there are a multitude of reasons. Development of biological control methods really blossomed after synthetic chemical pesticide application became the dominant method of pest control. Use of biological control grew due to practical needs to find a solution to pest problems when chemical pesticides

did not work or were not appropriate for controlling specific pests. Another major impetus for using biological control has been the fact that chemical pesticides can cause negative side-effects, leading to concerns about human health and the health and preservation of the environment. Biological controls leave no chemical residues and are usually quite host specific, especially in comparison to synthetic chemical pesticides.

As years have passed and scientific research has advanced, the types of approaches available for pest control have also increased in number and complexity. Within the field of biological control, a diversity of natural enemies can be used in many different ways. Other advances have been the ability to synthesize the active compounds used by pests for communication (pheromones), which are then used for controlling those same pests. An example of a relatively new type of control involves the fungus *Myrothecium verrucaria*, which produces multiple compounds that negatively affect plant parasitic nematodes. The fungus is mass-produced and then killed. The active compounds produced by the fungus are then applied to the soil to create an inhospitable environment for the nematodes.

Alternatively, the genes responsible for producing compounds that control pests have been moved into other organisms where they are expressed for production of pesticidal compounds in the specific areas where they are needed. The best known example is genetically engineered, or transgenic, plants. Genes that are currently used extensively for expression in plants encode production of a bacterial toxin (originally derived from *Bacillus thuringiensis*) that kills insects.

Based on our definition, use of only the compounds produced by natural enemies would not be called biological control. Use of these applications could instead be included in the larger categories of biologically based pest management or biorational pest control. However, disagreements over use of this terminology are far from resolved. Controversy centers around whether the organisms used for “biological control” must be living or just the source of compounds and genes. The following discussion describes the rationale underlying the definition of biological control as exploiting living organisms for the control of pests.

2.1.1 Is use of plants expressing Bt toxins biological control?

The bacterium *Bacillus thuringiensis* (Bt) is used extensively for control of arthropods due to its high virulence, low cost, ease of application, and narrow host specificity. Yet, the activity of Bt is due to toxins produced by this bacterial species. It has been argued that because the activity is due only to a toxin and not the living organisms, use of Bt should not be called biological control (Garcia *et al.*, 1988). In particular, this argument would encompass use of Bt-transgenic crops in which the toxin is expressed and no living organism is used (beside the crop plant). Based on the 1919 (Smith, 1919) definition of biological control, one of the original descriptions, biological control agents (including parasites, predators, and pathogens) should

provide self-sustained control with density-dependent responses to host populations (see Chapter 6). With foresight, as the use of biological control expanded, a more all-inclusive definition was drafted by DeBach (1964b) to include the activity of all parasites, predators and pathogens that decrease another organism's populations, not only during density-dependent relationships.

A definition published by the US National Academy of Sciences in 1988 expanded DeBach's definition to "the use of natural or modified organisms, genes or gene products to reduce the effects of undesirable organisms (pests), and to favor desirable organisms such as crops, trees, animals, and beneficial insects and microorganisms." Some biological control experts embrace this expanded definition as a means toward growth of biological control through adoption of new technologies (Cook, 1993; Charudattan *et al.*, 2002). Others worry that the expanded definition including genes and gene products could tarnish the positive image of biological control embraced by an environmentally aware public who might not welcome genetic engineering. Yet others feel that the recent additions to the definition lose the original aspect of interactions among populations of organisms (Perkins & Garcia, 1999). A solution to this controversy appears to have come in the use of the terms "biologically based pest management" or "bio-rational pest management," which include products from living organisms as well as the living organisms themselves. Use of these alternate terms to retain the emphasis of reliance on biological interactions while preserving the definition of the already otherwise-defined term "biological control" circumvents these problems.

2.2 | Natural control

The concept of a "balance of nature" has been traced to ancient times, when it was considered that numbers of each species were virtually constant. It was thought that each species had a role and place and extinction did not occur because it would disrupt the balance and harmony of nature. Outbreaks of species were often considered aberrations, having something to do with gods punishing humans for wrongdoing. Only after Darwin's time did early ecologists begin trying to understand how the "balance of nature" was attained and maintained.

Populations of the majority of species in nature are thought to be under naturally occurring regulation through complex interactions within food webs, and the majority of these species therefore do not increase to compete with humans. The problems historically addressed by biological control come from pestiferous species that have evaded the web of natural controls restricting their numbers. The goal of several different biological control strategies is to re-establish this level of self-sustained natural control, either through introducing a natural enemy for permanent establishment (classical biological control), or by altering the environment to conserve or enhance natural

enemy populations (conservation biological control). While the third major strategy, augmentation, is more immediate and is not aimed at establishing long-term regulation of pest populations, the augmentation strategy relies on these same basic interactions of natural enemies living at the expense of hosts. In fact, classical biological control introductions have been likened to “ecological experiments on a grand scale,” and illustrate both the “escape” of pest species relieved of natural enemies and their demise when enemies are restored to the system (Strong *et al.*, 1984).

To what extent successful biological control compares with the natural control regulating naturally occurring populations has been questioned. It seems that biological control often succeeds by establishing a single strong association between pest and natural enemy, a more simplified type of regulation than often would be seen in nature. Simple or not, the basic tenet behind use of classical and conservation biological control is that “natural control” can be used to reduce the pest population. Ecologists have worked for many years investigating the interactions between pests and their natural enemies to understand what is necessary for establishing, re-establishing, or maintaining natural control.

2.3 | Diversity in biological control

Biological control differs significantly depending on whether the pests are invertebrates, vertebrates, plants, or microorganisms. For biological control of invertebrates, hosts are usually small and sometimes mobile (at least in some life stages). Emphasis has been on plant-eating arthropods and arthropods of importance to public health. Virtually all natural enemies used for biological control of arthropods kill pests directly. Mortality of the pest is often very quick with predators but there can be a time lag with parasites or pathogens because they often first develop using the hosts as food before killing them.

Among pestiferous weeds, pests range from small herbs to large trees; these are stationary and, at times, dense. Biological control of weeds requires many individual natural enemies to damage a weed, unless the natural enemy attacks the so-called “Achilles heel” for that plant species (e.g., a part of the plant or its life cycle that is especially vulnerable), in which case fewer individuals could be necessary. Mortality of the weed is always delayed, if the plant dies at all, although growth and seed production would be reduced more quickly. Also, in contrast to biological control of arthropods, weeds do not move except through seed dispersal so herbivorous natural enemies generally do not have as much difficulty locating their target pests. Weedy plants can “escape” from a natural enemy through establishment of a disjunct population by means of long distance seed dispersal, but finding new isolated plant populations is often less of a problem for weed-feeding natural enemies compared with the difficulty for

arthropod-attacking natural enemies of finding and attacking mobile arthropod pests. Weeds are also different from arthropods as pests because competition with other plants can be important in mediating the outcome of biological control. If weeds can be partially suppressed by herbivory or disease then the weed can more easily be outcompeted by other plants that are hopefully desirable.

For the microorganisms causing plant disease, biological control is due to multitudes of microbial antagonists that compete with multitudes of plant pathogenic microbes. Both plant pathogens and their antagonists are usually tightly linked with specific habitats. For many programs, antagonists are applied preventively, so time before control is effective is not an issue.

Scientists working to control these diverse pests must adopt very different tactics with relation to the importance and immediacy of the pest problem, the type of impact on the pest that is needed, and the ability of both natural enemy and host/prey to disperse.

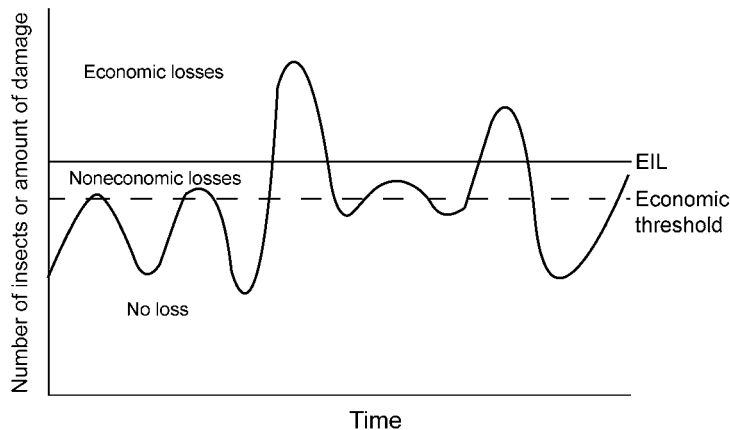
2.3.1 Is biological control always appropriate?

Biological control is principally used to combat arthropod pests, weeds, and plant pathogens and only in a few instances has biological control been used to control vertebrate pests (see Chapter 11). It has been used extensively for terrestrial systems, both above-ground and in the soil, starting out with uses in agriculture and forestry and later being applied to natural ecosystems to control invasive as well as native pests. Natural enemies have been successful in controlling arthropods and weeds in freshwater ecosystems, principally when used in contained bodies of water. Natural enemies are not presently being used in marine ecosystems but, given the growing number of invasives in this ecosystem, this possibility has been discussed (Lafferty & Kuris, 1996). However, there are certainly some types of pests and conditions for which biological control might not be the most appropriate type of control. These conditions include situations where pests must be totally eliminated very rapidly. Several such examples are described below.

Economic injury level of a crop

Presence of an organism in association with humans or some valued resource does not always mean that the organism needs to be controlled. This is particularly true with agricultural pests or pests that can be present at low densities without causing problems. A concept that has been developed to determine whether an organism needs to be controlled is called the economic injury level (EIL). The economic injury level is defined as the lowest density of pests that will cause economic damage (Pedigo, 1996). Actually, an economic threshold is generally set below the economic injury level and, once densities of a potential pest reach this threshold, control practices should begin (Fig. 2.1). If managers wait until pest densities reach the economic injury level, the pests are sure to increase over that density and cause economic loss before being controlled.

Fig. 2.1 Relationship between the density of an insect population, the economic threshold used to trigger management decisions and the economic injury level (EIL), above which economic loss occurs.



Of course, economic injury levels are very dynamic and differ based on crop value, management costs, degree of injury, and crop susceptibility to injury. Among these factors, crop value is perhaps the most notorious for fluctuations and unpredictability. Not all pest problems are amenable to economic analysis so it can be difficult to determine how much money is lost due to activity of the pest. Therefore, use of an economic injury level is most suited for pests of agricultural crops. While the actual economic injury level can change each year to some extent, this relationship clearly demonstrates that control is not always necessary, and sampling the pest population should be used when it is necessary to determine whether to control pests.

The economic injury level of the crop being managed is important when considering use of biological control. Some crops, such as cut flowers, can withstand little damage without monetary losses, and the economic injury level of these crops is set very low; few to no pests can be present before economic losses are incurred. Biological control that could be used on this type of crop would need agents that act very quickly to kill all pests, such as fast-acting biopesticides applied inundatively. For crops that can tolerate the presence of some pests before economic injury occurs, there is more flexibility in the types of biological control that could be used. Many natural enemies such as parasitoids and pathogens take some time before killing hosts, and for crops with a higher economic injury level, such as pests feeding on the foliage of greenhouse vegetables, presence of pests for some period before death would not always ruin the product. For long-term biological control of established pests in the field, a system with a higher economic injury level is often thought to be necessary so that some pest population is present to maintain a natural enemy population in the area. Then, if the pest population increases, the natural enemies will be present so they can respond more quickly than if they were absent and needed to recolonize the site.

Host density

Natural enemies require time to act, including time to find hosts and time to kill or disable hosts. If pest populations are already at

outbreak densities when the decision to undertake control is made, most types of natural enemies would not be able to respond quickly enough to completely prevent further damage. Therefore, in many systems, use of natural enemies is not considered appropriate for extremely high densities of pests (often called outbreaks).

Exceptions would be use of fast-acting biopesticides, which act like chemical pesticides in their rate of response or, for some agents, application of very high doses. Another exception would be natural enemies being introduced for permanent establishment and long-term control, immediate control never having been expected. Nevertheless, natural enemies are usually best at managing pests at lower densities and are not always appropriate for immediate control of outbreak densities of pests.

Eradication

When an invasive species is introduced to a new area, governmental bodies frequently decide to eradicate it, that is, totally eliminate it from that area. Eradication programs are often large undertakings. Some evaluations of eradication programs have demonstrated that eradication is very difficult and rarely possible (Dahlsten & Garcia, 1989). Because eradication programs are usually focused on rapid action, slower-acting types of biological control are certainly not appropriate. Eradication campaigns usually employ fast-acting and lethal pest control agents. While chemical pesticides are usually the main types of control used, in several recent examples natural enemies were used because the pests targeted for eradication occurred in urban areas. Repeated aerial sprays of the insect pathogenic bacterium *Bacillus thuringiensis* were used in British Columbia, Canada, to eradicate the Asian gypsy moth (*Lymantra dispar*, Asian strain) in 1992 and in Auckland, New Zealand, to eradicate the white-spotted tussock moth, *Orgyia thyellina*, in 1996–98 and the painted apple moth, *Teia anartoides*, beginning in 2002.

2.4 History of biological control

The first records of biological control describe habitat manipulation to increase natural enemy populations. As early as 324 BC, people in China encouraged populations of the ant *Oecophylla smaragdina* in citrus trees to control caterpillars and large boring beetles. This species of ant builds large paper nests in trees resulting in legions of ants inhabiting the trees. Colonies could be purchased or were moved from wild trees into orchards. In addition, to foster movement of ants within the orchard, bamboo runways were placed between trees. Surprisingly, these practices were still seen in the Shan States of North Burma in the 1950s. In 1775, a similar practice was reported from date growers in Yemen, who moved colonies of predatory ants from the mountains to date groves to control pest insects (DeBach & Rosen, 1991).

These earliest uses of natural enemies to control pests involved manipulations of pre-existing natural enemies visible to the naked eye that were generalist predators feeding on many types of prey. With scientific advances, other groups of natural enemies that were smaller began to be investigated and then considered as control agents. The fact that smaller invertebrates live as parasites of larger invertebrates was first reported in the 1600s. With the invention of the microscope by van Leeuwenhoek in the late 1600s it became possible to learn more about these ever-smaller natural enemies. Although microorganisms had been seen previously, it was not until 1835 that microorganisms were first shown to be the cause of disease by Agostino Bassi, working with the fungal pathogen *Beauveria bassiana* infecting silkworm, *Bombyx mori*, larvae. In 1874, W. Roberts, working with the fungus *Penicillium* and bacteria, first demonstrated that microorganisms could inhibit one another and, in 1908, M. C. Potter first demonstrated such inhibition among plant pathogenic microorganisms (Baker, 1987).

As European explorers set out to discover new lands and establish trading colonies, movement of humans around the world became possible. Movement of plants that could be used as crops followed and pests were often accidentally introduced with the crops. In some cases, organisms that were familiar to colonists from Europe were purposefully introduced, only to become pests, as with rabbits introduced to Australia. It was frequently found that organisms virtually unknown in their areas of endemism could become major pests in areas where they had been introduced, and it became commonly accepted that this was due to their release from control by natural enemies (the “enemy release hypothesis”). This hypothesis states that a pest is able to increase to high densities due to the absence of the natural enemies that regulate populations of that pest in its area of endemism.

As practices in agriculture and forestry for producing crops improved, single cultivars were grown in ever-larger monocultures. These changes were accompanied by greater pest problems due to both native and introduced pests. With such pest problems, the world was ripe for accepting synthetic chemical pesticides when they were developed. The synthetic chemical insecticide DDT and the synthetic chemical herbicide 2,4-D first began to be tested and used for pest control around 1942, and development and use of a great diversity of pesticides followed. Although natural enemies had been discovered and described much earlier, developments in the use of natural enemies for control only seriously diversified and escalated after problems with DDT became evident.

Of course scientists had been thinking of using natural enemies for pest control long before the advent of synthetic chemical pesticides. Even Linnaeus suggested using predatory insects to control insect pests in 1752 (US National Research Council, 1996). The term “biological control” was coined in relation to plant pathogens by C. F. von Tubeuf in 1914 and then applied to insects by H. S. Smith in 1919 (Baker, 1987). While similar basic principles underlie much of

biological control, control of different groups of pests evolved quite separately. Scientists working with these different groups of pests and different groups of natural enemies need specific training. Scientists trained as entomologists generally specialized either in predators and parasitoids for controlling arthropods or, with backgrounds in plant science and entomology, in phytophagous arthropods for use against weeds. Knowledge of microbiology, plant science, and plant pathology is necessary for plant pathologists working to control plant pathogens or to control weeds with microbes and knowledge of both microbiology and entomology is required to work on pathogens for control of arthropods. As biological control grew, it became evident that the diverse array of pest control problems would require a variety of biological control strategies. Scientists working to control arthropods, weeds or plant pathogens historically had few opportunities for interchange although they certainly communicated results within each subdiscipline. The different subdisciplines thus developed their own definitions and practices.

In more recent years, there has been an attempt toward fostering communication among practitioners working in these different areas of biological control. Notably, several books published in the last decade are cross-disciplinary in scope (Lumsden & Vaughn, 1993; Hokkanen & Lynch, 1995; Van Driesche & Bellows, 1996; Bellows & Fisher, 1999; Gurr & Wratten, 2000). The goal of the scientific journal of the International Organization for Biological Control, named *BioControl* (previously *Entomophaga*), is to publish scientific research from all different branches of biological control. In 1991 two new journals, *Biological Control: Theory and Application in Pest Management* and *Biocontrol Science and Technology*, were begun specifically to publish research results from across all types of biological control research.

Due to the independent growth of the different subdisciplines, the specific histories of each will be presented separately.

2.4.1 Controlling arthropod pests

Before the advent of restrictions on movement of organisms around the world, pest introductions were numerous and frequently caused dramatic outbreaks. The cottony cushion scale (*Icerya purchasi*), an insect attacking citrus, was introduced to southern California where it caused enough damage in the mid-late 1800s to threaten the existence of the California citrus industry. A predatory lady beetle (*Rodolia cardinalis*) and a parasitic fly (*Cryptochaetum iceryae*) were introduced from Australia, the original home of the scale insect. These introductions led to phenomenal success and brought public attention to biological control (see Box 3.1). For a period following this success, there were many introductions of predatory and parasitic insects around the world to control introduced pests, particularly lady beetles to control aphids and scale insects, but no programs were as successful. This period of seemingly haphazard introductions following the cottony cushion scale success was considered a little too enthusiastic by

some, who later called this period the “lady bird [lady beetle] fantasy” (Greathead, 1994).

Introduction of exotic natural enemies to control introduced pests has remained very active. After DDT first became available, classical biological control programs continued to be undertaken at an increasing rate by entomologists trying to repeat the success with cottony cushion scale. However, this control strategy was not as successful in the 1950s, although many introductions were made. This increased rate at which classical biological control organisms were released was possibly because scientists were trying to compete with chemical pesticides. Without extensive background information, releases were on a “try it and see basis,” hoping for quick success (Greathead, 1994). In 1983, Howarth published his first article criticizing the non-target effects of introductions of exotic natural enemies, especially regarding classical biological control of insects and weeds (see Chapter 18). The rate of introductions against exotics has slowed since then, with increased emphasis on non-target testing. However, use of classical biological control has not stopped and this strategy remains the best option for specific pest situations.

As use of biological control grew, practitioners began investing more effort using natural enemies in ways other than classical biological control. In England in 1895, the egg parasitoid *Trichogramma* became the first natural enemy to be mass produced for release to control pest arthropods. The ability to mass produce parasitoids and predators was subsequently developed but was not used extensively until the 1970s, when use of mass-produced natural enemies in greenhouses escalated. This type of augmentative use of natural enemies has increased exponentially since then.

Work with pathogens to control arthropods began in earnest later than work with predators and parasitoids, in part because scientific advances were necessary to be able to work easily with microbes. While biological control introductions with arthropod natural enemies were made in North America against cottony cushion scale as early as 1886–87, it was not until the twentieth century that scientists understood how viruses worked. Pathogens began being developed to be used as formulated biopesticides so that the numbers of organisms released could overcome the lack of dispersal by most pathogens. In 1948, a bacterial pathogen for control of Japanese beetles was the first insect pathogen registered for control in the USA. As will be described, the number of arthropod pathogens used has increased to fulfill the specific needs of different systems.

Today, use of natural enemies to control arthropods is usually part of integrated pest management programs (IPM). The concept of IPM was proposed in 1959 (see Chapter 19), and its adoption has increased since then, both in response to systems in which pesticides are not effective or cannot be used and systems where use of natural enemies for control is preferred. Conservation or enhancement of the resident natural enemies of arthropods for control is also included as part of IPM programs.

2.4.2 Controlling weeds

As stated by Goeden & Andrés (1999), “Like so many other aspects of science, [the study of biological control of weeds] began by accident.” In 1795, a scale insect called cochineal that was cultured commercially as a source of carmine dye, was introduced from Brazil to northern India for dye production. However, the species that was introduced was not the superb dye-producer *Dactylopius coccus*, but by accident, it was a related species, *Dactylopius ceylonicus*. Instead of reproducing well on the spineless prickly pear grown specifically for the dye production, *D. ceylonicus* moved onto its natural host plant the prickly pear *Opuntia vulgaris* that had been introduced to northern India and had become a problematic weed. The value of *D. ceylonicus* as a control agent was realized and from 1836 to 1838, this species was introduced to southern India and then in the 1860s to Sri Lanka. In both areas, *D. ceylonicus* provided successful control of the weedy *O. vulgaris*.

Classical biological control of weeds grew and was used in numerous countries with the principal emphasis being use of herbivorous insect natural enemies to control introduced, perennial weeds in relatively undisturbed areas such as rangelands. This changed in the late 1950s and early 1960s when programs were initiated against aquatic and semi-aquatic weeds, annuals, biennials, and weeds growing in croplands, along roadsides and invading natural ecosystems. The diversity in types of weeds to control and types of natural enemies to use for control continues increasing today.

The second type of approach, use of plant pathogens as bioherbicides for mass application and more immediate weed control, began around 1971. Research in the late 1960s through the 1980s resulted in registration of two plant pathogens as bioherbicides, and these have been used in agriculture for the past two decades (Roskopf *et al.*, 1999). Research and development of plant pathogens as bioherbicides is an active field today.

2.4.3 Controlling plant pathogens and plant parasitic nematodes

Biological control of plant pathogens got its start much later. Because this field is based totally on microorganisms, more technically advanced techniques were required for its growth. The first biological control strategy that was used extensively against arthropods, classical biological control, was not appropriate against plant pathogens. Early in the 1900s, plant pathologists recognized that microorganisms could suppress plant disease and this activity could be manipulated through cultural and management practices (Cook & Baker, 1983). The first trials attempting to suppress plant disease by adding beneficial microorganisms to soil occurred in the 1920s. It was not until the 1950s that the first biological control organism was commercially used to control infection of cut tree stumps by *Heterobasidion annosum*, a fungal pathogen that has the potential to spread through

root grafts to healthy trees nearby. A second highly successful product was developed for control of crown gall in the 1970s. Biological control of plant pathogens and plant parasitic nematodes was destined to continue to grow through development of biopesticides, especially against pathogens in the soil environment. By 1995, 30 different biological control organisms were available as commercial formulations for suppression of plant diseases. By 2000, the number of biological control organisms had not changed substantially but they were being marketed under 80 different product names (Whipps & Davies, 2000). Few of these newer products have been on the market for more than 10–20 years.

Soils in some regions were identified as suppressive or conducive to plant pathogens of banana as early as 1922. Naturally suppressive soils have now been identified for numerous crops and plant pathologists are working on understanding the mechanisms involved in suppression and thus developing ways to create suppressive soils. There is a great need for biological control to control soil-dwelling plant parasitic nematodes and, at present, few biological control products are available.

Scientists from a diversity of backgrounds have worked to develop methods for biological control of animal, plant, and microbial pests and their findings have been reported and summarized in numerous books. More-recent books presenting broad coverages of biological control are listed at the end of this chapter. Although not all can be listed here, many additional excellent books are narrower in scope and cover biological control of specific types of pests, specific types of natural enemies, different types of resources to protect, or biological control in different geographic regions.

2.5 | Studying biological control

Use of biological control requires much more background information about the biology and ecology of pests than use of chemical pesticides. For all types of biological control, it is necessary to demonstrate that natural enemies are effective at controlling pests. Methods have been developed in ecology for evaluating the importance of natural enemies throughout the life of a host or prey species. Life tables are used to document the effects of natural enemies on pest populations of different ages. This type of analysis is easier to use with insects that have discrete life stages, such as egg, different larval instars, pupae and adults. Mathematical models can then be used to explore interactions or suggest hypotheses about what regulates population densities. However, these types of analysis do not really demonstrate the efficacy of natural enemies. Experimental methods are needed to show this.

There are numerous ways the effects of natural enemies can be demonstrated. The following discussion will include methods used for

evaluating biological control of arthropods (Luck *et al.*, 1988) but comparable methods are appropriate for documenting success of other types of natural enemies against pests. For all of these experimental methods, the emphasis is on documenting a difference in host/prey populations when natural enemies are absent versus when they are present.

2.5.1 Sampling

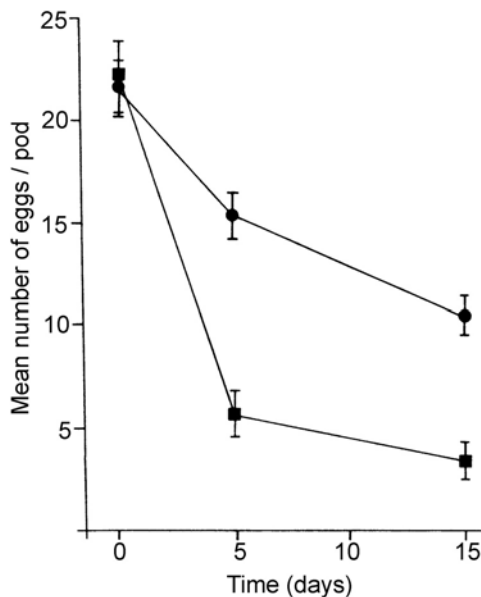
Quantification of pest densities before and after natural enemy release has often been used to demonstrate that natural enemies have been effective at suppressing pest populations. Percentage infection, parasitism, and predation have also been used to demonstrate that natural enemies have been responsible for pest control. However, the important information regarding whether control was achieved is the absolute number of surviving pests and not the percentage that survive. This is because even if pest populations were very high and there was a high percentage mortality, there could still be enough remaining individuals to cause significant damage. Critics of this approach have pointed out that some new pests can decrease in numbers when no biological control introduction has been made. Therefore, after introduction, densities should be simultaneously quantified in areas with and without the natural enemies. These techniques are pretty much standard for introductions of exotic natural enemies against introduced pests but are certainly appropriate for other types of biological control as well.

2.5.2 Cages

Cages have been used to evaluate effects of natural enemies more frequently than any other method. Cages can exclude natural enemies from a segment of the host or prey population and subsequent differences in densities between the wild population and the protected population can be used to indicate the effect of the natural enemies. For example, such techniques were used to evaluate the impact of predators on cereal aphid populations. It was shown that the caged populations increased at a far greater rate than the uncaged populations. The caged population was protected from natural enemies while the uncaged population was exposed to natural enemies in the field. Cages do not have to completely enclose the prey but only exclude the natural enemies of concern, as in the case of exclusion of predatory ants using sticky bands (Fig. 2.2). Alternatively, cages are used for including natural enemies with hosts so that both natural enemies and hosts can be sampled. This is especially useful for natural enemies with mobile hosts that might be difficult to locate in significant numbers once a study begins. For example gypsy moth larvae (*Lymantria dispar*) will frequently feed on foliage high in tree canopies but can be caged on lower branches during experiments so that groups of insects can be repeatedly observed.

Results from caged studies must be interpreted with caution because it is extremely difficult to make conditions within cages

Fig. 2.2 Mean bruchid beetle eggs per seed pod of *Acacia farnesiana* on control and protected branches at days 0, 5, and 15, after branches were protected from predatory ants by wrapping a 10 cm wide band of tape around the base and applying sticky material to the tape. (Traveset, 1990.)



realistic. Densities of hosts and natural enemies can be unrealistic because dispersal is not possible, encounter rate between natural enemy and host could be artificially elevated, behavior of the host or its natural enemy can be affected by the cage itself, and the cage can create an interior microclimate different from the area outside of the cage.

2.5.3 Removal techniques

An effective method used for removal of natural enemies to evaluate their effect is the “insecticidal check method.” This method has been used primarily to study the effect of insect natural enemies but would also be applicable for studying microbes that can be killed with pesticides. An area is sprayed with selective insecticides to exclude insect natural enemies, and pest densities in this area (either arthropod pests or weeds) are later compared with controls where no treatments were applied (Fig. 2.3). This method assumes that the pest population will not be totally eradicated by the spray but the natural enemy population will be totally killed or so sparse that it will not increase rapidly. This is not a bad assumption in some cases, for example when sprays are made early in the season and natural enemies are present but hosts/prey are in a more resistant stage, and in such cases this method is very appropriate. However, there can be problems interpreting results if the pesticide affects the treated area in ways other than killing the natural enemies. For example, spraying sublethal doses of pesticides on some phytophagous mites can stimulate their reproduction and sometimes lead to greater production of females. In these cases, spraying pesticides would bias results from the study,

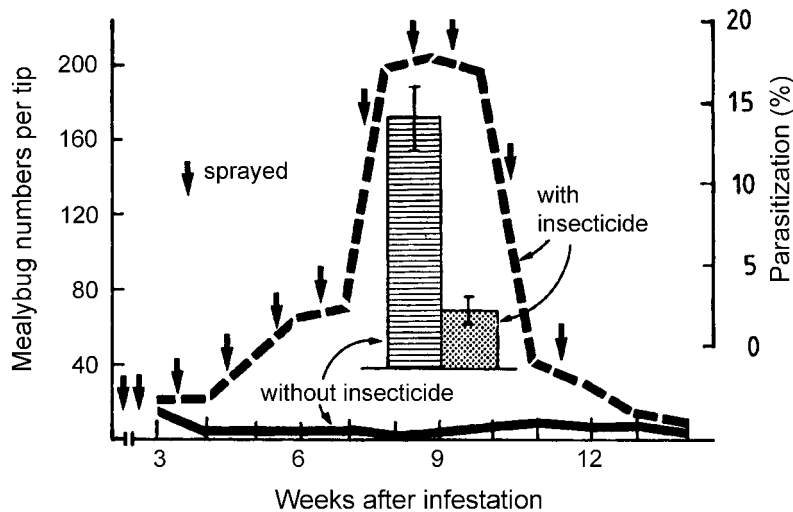


Fig. 2.3 Cassava mealybug, *Phenacoccus manihoti*, population development in insecticide-treated and untreated plots, with the mean levels of parasitism. (Neuenschwander & Herren, 1988.)

demonstrating artificially enhanced pest populations in the sprayed area.

2.5.4 Prey enrichment

Adding prey or hosts to the field can be used to test the efficacy of natural enemies. This technique is especially useful for testing non-mobile stages, such as eggs or pupae. This is also a great technique for studying hosts that would be difficult to find in the field, such as soil- or tree-dwelling pests. A common technique for studying entomopathogenic nematodes in the soil is to place larvae of a very susceptible species, the wax moth, *Galleria mellonella*, in the soil and later retrieve them to evaluate the percentage of larvae found and killed by nematodes. As with studies using cages, interpretation of data from enrichment studies can be difficult because in most instances the hosts and prey placed in the field might not be found exactly at that time or place or in that density under natural conditions.

2.5.5 Direct observation

This technique is useful for predators or parasitoids that can be observed attacking hosts. It is especially very difficult to determine to what extent predators have been the cause of declining pest populations. In many cases the predator eats the entire prey item and leaves no evidence behind of its meal, thus, this event cannot be counted in the field. While direct observation is simple, it requires a huge time commitment and cannot be used if the predator or prey are cryptic or are easily disturbed, or if the predator consumes the prey very quickly. However, direct observation was used successfully to monitor the numbers of green rice leafhoppers (*Nephotettix cincticeps*) preyed upon by four species of spiders, with part of the observations taking place at night using flashlights because it turned out that predation was primarily nocturnal (Kiritani *et al.*, 1972).

2.5.6 Evidence of natural enemy activity or presence

This method involves having some way to evaluate whether natural enemies have attacked specific prey. In some systems, natural enemies leave evidence of killed hosts or prey and this can be used to quantify activity. For example, mice feeding on gypsy moth pupae characteristically leave behind an empty pupal case different in appearance from pupal cases that have been attacked by ants, another major predator. Parasitoids can leave behind their pupal cases next to cadavers of hosts and the different parasitoid puparia are characteristic of different species. Cadavers of arthropods killed by pathogens often remain in the field for some period of time, during which they can be sampled. However, in many cases, such helpful evidence is lacking and no indication of prior presence of a pest is left.

Laboratory-bench assays have been developed for evaluating the activity of natural enemies. Predators can be collected in the field and their gut contents can be evaluated to determine what they have eaten. The most widely used techniques are based on developing vertebrate antibodies to specific prey and then testing predators caught in the field to evaluate whether the antibodies react to their gut contents. These general types of tests are called immunoassays and methodology is similar to methods used in medical laboratories. If the antibodies react, this indicates that the predator had eaten that prey species. Accuracy in detection depends on the size of the prey, the size of the meal, the time since the meal, the means of feeding (sucking versus chewing), occurrence of closely related prey (which might also cause a reaction), and the sensitivity of the test. Electrophoresis can also be used to detect the presence of prey protein in the guts of predators.

Instead of developing antibodies or using electrophoresis, prey tissues can be marked using a variety of materials, including radioactive isotopes, rare elements such as rubidium, or dyes (e.g., fluorescent dyes). Suspected predators are then collected and assayed for the marker.

If pests are sampled from the field and percentage parasitism or infection is to be determined, insects must be reared. However, rearing is seldom easy and introduces its own bias to the study if the hosts are stressed during rearing. If a technique such as electrophoresis, immunoassay, or marking prey is used to detect the presence of a parasitoid or pathogen within a host, rearing the hosts before analysis is often not necessary, thus avoiding potential problems introduced when rearing insects.

All of these molecular techniques require special training and equipment. However, there are distinct advantages because this methodology provides a different type of information than other studies. Also, these techniques are time efficient, which can be a concern when working in biological systems where critical time periods can be brief. Field-collected samples need only to be processed to a limited extent at the time of collection and often can then be stored for

evaluation later. Field work is generally very labor intensive for a defined period of time and the prospect of being able to process samples at a later date makes it possible to evaluate more samples and thus learn more about natural enemy activity.

FURTHER READING

- Graham, Jr., F. *The Dragon Hunters*. New York, NY: E. P. Dutton, 1984.
- Greathead, D. J. History of biological control. *Antenna*, **18** (1994), 187–199.
- Sawyer, R. C. *To Make A Spotless Orange: Biological Control in California*. Ames, IA: Iowa State University Press, 1996.
- Smith, R. F., Mittler, T. E. & Smith, C. N. *History of Entomology*. Palo Alto, CA: Annual Reviews, 1973.

SELECTED GENERAL REFERENCES ON BIOLOGICAL CONTROL

- Bellows, T. S. & Fisher, T. W. (ed.). *Handbook of Biological Control*. San Diego, CA: Academic Press, 1999.
- Campbell, R. *Biological Control of Microbial Plant Pathogens*. Cambridge: Cambridge University Press, 1989.
- Charlet, L. D. & Brewer, G. J. (ed.). *Biological Control of Native or Indigenous Insect Pests: Challenges, Constraints, and Potential*. Lanham, MD: Entomological Society of America, 1999.
- Cook, R. J. & Baker, K. F. *The Nature and Practice of Biological Control of Plant Pathogens*. St. Paul, MN: The American Phytopathological Society, 1983.
- DeBach, P. & Rosen, D. *Biological Control by Natural Enemies*, 2nd edn. Cambridge: Cambridge University Press, 1991.
- Flint, M. L. & Dreistadt, S. H. *Natural Enemies Handbook: The Illustrated Guide to Biological Pest Control*. Berkeley, CA: University of California Press, 1998.
- Franz, J. M. (ed.). *Biological Plant and Health Protection*. Stuttgart: Gustav Fischer Verlag, 1986.
- Gurr, G. & Wratten, S. (ed.). *Biological Control: Measures of Success*. Dordrecht, NL: Kluwer Academic Publishers, 2000.
- Hokkanen, H. M. T. & Lynch, J. M. (ed.). *Biological Control: Benefits and Risks*. Cambridge: Cambridge University Press, 1995.
- Jervis, M. & Kidd, N. (eds). *Insect Natural Enemies: Practical Approaches to Their Study and Evaluation*. London: Chapman and Hall, 1996.
- Lumsden, R. D. & Vaughn, J. L. (eds). *Pest Management: Biologically Based Technologies*. Washington, DC: American Chemical Society, 1993.
- Mackauer, M., Ehler, L. E. & Roland, J. (eds). *Critical Issues in Biological Control*. Andover, UK: Intercept, 1990.
- Mukerji, K. G. & Garg, K. L. (eds). *Biocontrol of Plant Diseases*, 2 vols. Boca Raton, FL: CRC Press, 1988.
- van den Bosch, R., Messenger, P. S. & Gutierrez, A. P. *An Introduction to Biological Control*. New York: Plenum Press, 1982.
- Van Driesche, R. G. & Bellows, T. S., Jr. *Biological Control*. New York: Chapman & Hall, 1996.
- Wood, K. R. & Way, M. J. Biological control of pests, pathogens and weeds. *Philosophical Transactions of the Royal Society of London*, B **318** (1998), 109–376.

Part I

Strategies for using natural enemies

Just as you find different types of pests with different attributes in different ecosystems, control strategies that are most appropriate for these different circumstances also vary. For example, the same goals and methods would not apply for control of mosquitoes inhabiting a long-lived forest on a nature preserve as for control of an aphid species in an agricultural monoculture of corn plants. In this example, the damage from the pests differs as mosquitoes are a public health problem and our tolerance to the presence of mosquitoes is usually low. Aphids attack plants and, if they aren't vectoring plant diseases, aphids must reach high population densities before they cause significant plant damage. The ecosystems in this example also differ because a nature preserve is more permanent while a corn crop in an agricultural field is temporary. Therefore, for the preserve, controls that operate over the long term could be appropriate, while for the monoculture that will be disrupted when harvested, permanence of control is often not important.

As interest in use of biological controls has grown, strategies for using biological control agents in very different ways to suit different needs have been developed. These vary in factors such as the source and types of natural enemies, whether natural enemies are released or resident natural enemies are manipulated and whether control is immediate or long term. Strategies can be grouped into four major categories: classical biological control, inoculative biological control, inundative biological control, and conservation biological control (Eilenberg *et al.*, 2001). This grouping follows the ideology of entomologists and was initially described relative to control of insects with insect natural enemies. Weed scientists, plant pathologists, and even some entomologists working in biological control, have published divergent ways for grouping and naming biological control strategies. Unfortunately, this diversity in terminology has decreased the ability to communicate among the different biological control disciplines

(i.e., biological control of animals, weeds, and plant pathogens), thus widening the gap among scientists working in these different yet related fields. In this book, the four-part system of thinking about strategies for use is applied throughout, striving toward some unification in the terminology used across the divergent fields within biological control. The following three chapters will provide definitions, descriptions and examples of uses of these different biological control strategies.

Chapter 3

Classical biological control

This strategy provided the first means developed on a large scale for using natural enemies for pest control, hence the name “classical” biological control. The term “introduction of new natural enemies” has also been used to refer to this same strategy, described below.

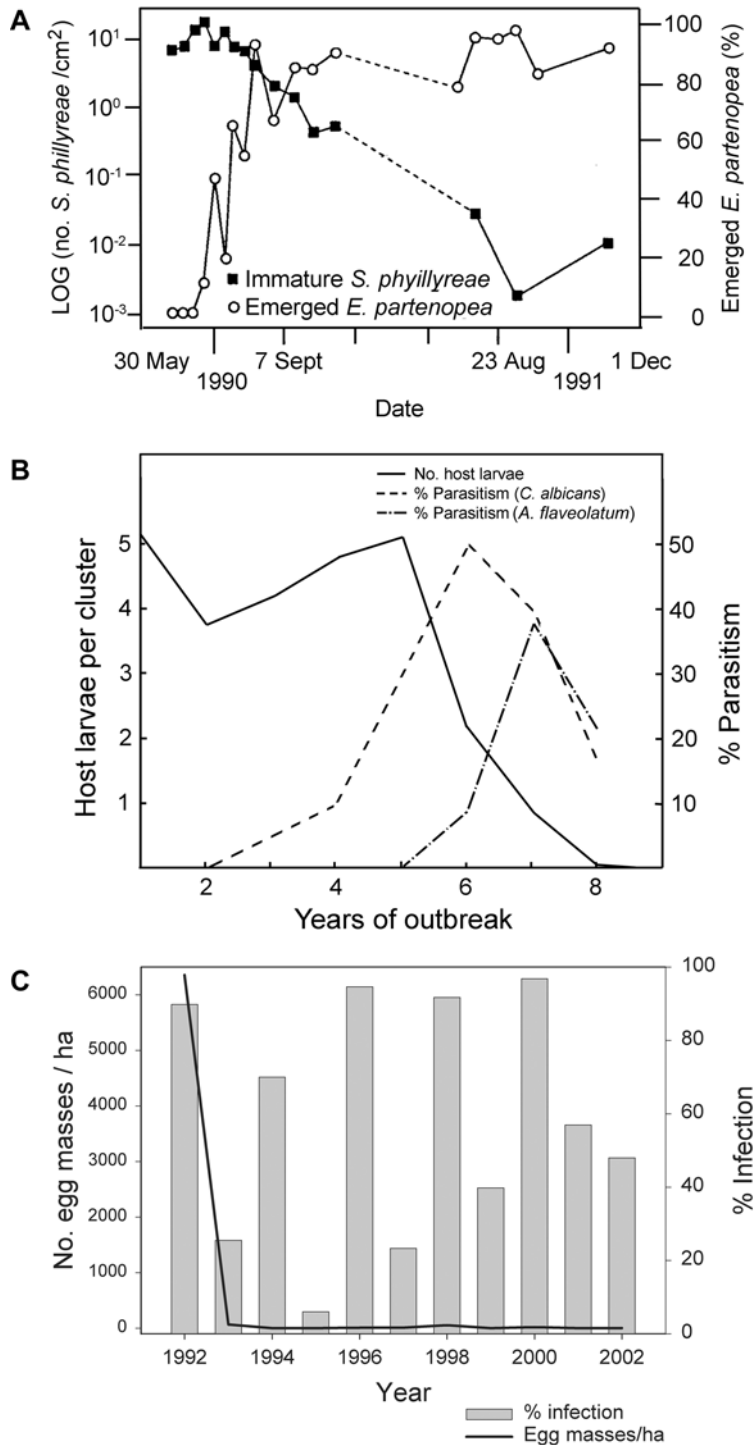
The intentional introduction of an exotic biological control agent for permanent establishment and long-term pest control

(Eilenberg *et al.*, 2001)

Importantly, the goal is quite specific: to release an exotic natural enemy into a new environment so that it will become established and will regulate a pest population over the long term without further intervention. Classical biological control has been used extensively and, as we will discuss, some programs have been extremely successful (Fig. 3.1). This strategy was initially developed to control introduced pests, based on the following scenario. Scientists noted that many introduced pests are not problematic in their areas of origin, where they are often controlled by a community of natural enemies. After introduction to a new area, in some cases the introduced species increases in number to become a pest. It is thought that the pest is able to increase because in the new area the natural enemies that would naturally regulate this species are not present. This basic assumption of classical biological control has been called the “enemy release hypothesis.” The goal with classical biological control is to re-establish the “natural balance” that controls the pest in its native habitat.

The dramatically successful release of the *Vedalia* beetle against the cottony cushion scale attacking citrus trees in California is often said to have launched the use of classical biological control (Box 3.1). Used against insect pests since the late 1800s, this strategy increased to almost 850 releases between 1960 and 1969 alone and classical biological control remains in use against insect pests today (Fig. 3.2A). By 2001, over 2100 species of insect predators and parasitoids had been released for classical biological control of almost 600 insect pests in over 200 different countries or islands around the world (D. Greathead, pers. commun.) (Table 3.1). For use of classical biological control

Fig. 3.1 A. After introduction of the parasitoid *Encarsia inaron*, changes in density of immature ash whitefly, *Siphoninus phillyreae*, and percentage of parasitized whitefly pupae (= emerged *E. inaron*) on pomegranate in California, 1990–91 (Bellows *et al.*, 1992). B. History of a winter moth, *Operophtera brumata*, infestation and parasitism by the tachinid *Cyzenis albicans* and the ichneumonid *Agrypon flaveolatum* in Nova Scotia. Winter moth was accidentally introduced and both parasitoids were introduced for classical biological control. Data are from seven different areas and time is the number of years the winter moth outbreak persisted, beginning one year before *C. albicans* appeared in the population (Embree, 1966). C. Changes in density of introduced gypsy moth, *Lymantria dispar*, after the fungal pathogen *Entomophaga maimaiga* moved into central New York State. (A. E. Hajek, unpublished data.)



Box 3.1 | Introducing the Vedalia beetle against cottony cushion scale

In California in 1868, the cottony cushion scale was a new pest attacking citrus, pear, and acacia in southern California. By 1880, it had spread all over California and was seriously damaging citrus orchards wherever it occurred. In 1886, frustrated growers were pulling out or burning citrus trees because they couldn't control this pest as it lay waste to their orchards and land values plummeted. Entomologists guessed that the scale was from Australia, the country from which much of the citrus had been imported. The head of entomology for the US government, Charles V. Riley, requested that someone be sent to Australia to search for natural enemies. However, this request was turned down due to a restriction on international travel for employees of the Division of Entomology. However, in 1888, Albert Koebele was sent to search for natural enemies in the guise of attending the International Exposition in Melbourne. In actuality, Koebele barely attended the meeting and instead traveled throughout Australia searching for natural enemies for this project. Even with the assistance of Australian entomologists, it took a while for Koebele to



Portrait of Albert Koebele, the entomologist who collected the Vedalia beetle from Australia to import to California for control of the cottony cushion scale. (Swezey, 1943.)

locate cottony cushion scales. The most promising natural enemies Koebele found were a parasitic fly and a lady beetle (Coccinellidae), *Rodolia cardinalis* (although at the time that Koebele found it, this beetle was known as *Vedalia cardinalis*, hence the common name that has persisted).



Vedalia beetle (*Rodolia cardinalis*) adult and larvae with a wax-covered cottony cushion scale, *Icerya purchasi*. (Photo by Jack Kelly Clark, courtesy University of California Statewide IPM Program.)

Koebele collected and sent both the flies and beetles in five shipments, during which both scales and natural enemies had to be kept alive throughout the 3-week boat trip from Australia to California. It is no small feat keeping organisms alive during transit today and the obstacles faced to keep citrus trees, scales, and natural enemies alive for three weeks on the open ocean were substantial. During one voyage, the shipments were maintained in an ice house on board the ship but, during a gale, the parcels fell off shelves and were crushed by cakes of ice falling on them. Despite these difficulties, by 1889, a total of 514 individuals of *R. cardinalis* had arrived in California. These beetles were released, and 4 months after the first release, adult Vedalia beetles were swarming over a 3,200 tree orchard that had previously been heavily infested with scale. To hasten spread of the beetles, branches covered with scale-feeding beetles were transported to uninfested orchards. By 1890, all infestations of the cottony cushion scale were completely decimated, the citrus industry was saved, and the total control program had cost less than \$5,000, including salaries. The citrus industry has reaped benefits of millions of dollars annually ever since due to control of cottony cushion scale. The delighted Californians honored Mr. Koebele by giving him a gold watch and his wife received a pair of diamond earrings.

When Koebele was searching for natural enemies with the Australian scientists helping him, for a while he thought that the fly he had collected would be the more important of the two natural enemies. The fly, *Cryptochaetum iceryae*, became established after releases of 1,200 individuals and, in fact, is the major agent controlling cottony cushion scale along the California coast. This example shows just how difficult it is for researchers to judge how successful a specific natural enemy will be; in this case, the Vedalia beetle turned out to be astoundingly effective while the favorite, *C. iceryae*, was also successful but over a smaller area.

The Vedalia beetle today continues controlling the cottony cushion scale in the interior of California. The scale can still be found and can even increase in abundance if pesticides are used in orchards so that beetles and flies are killed. In such cases, natural enemies are reintroduced and control is once more established. In addition, these beetles and flies have been introduced in numerous other countries around the world, in the wake of the success of the introductions against the cottony cushion scale (DeBach & Rosen, 1991).

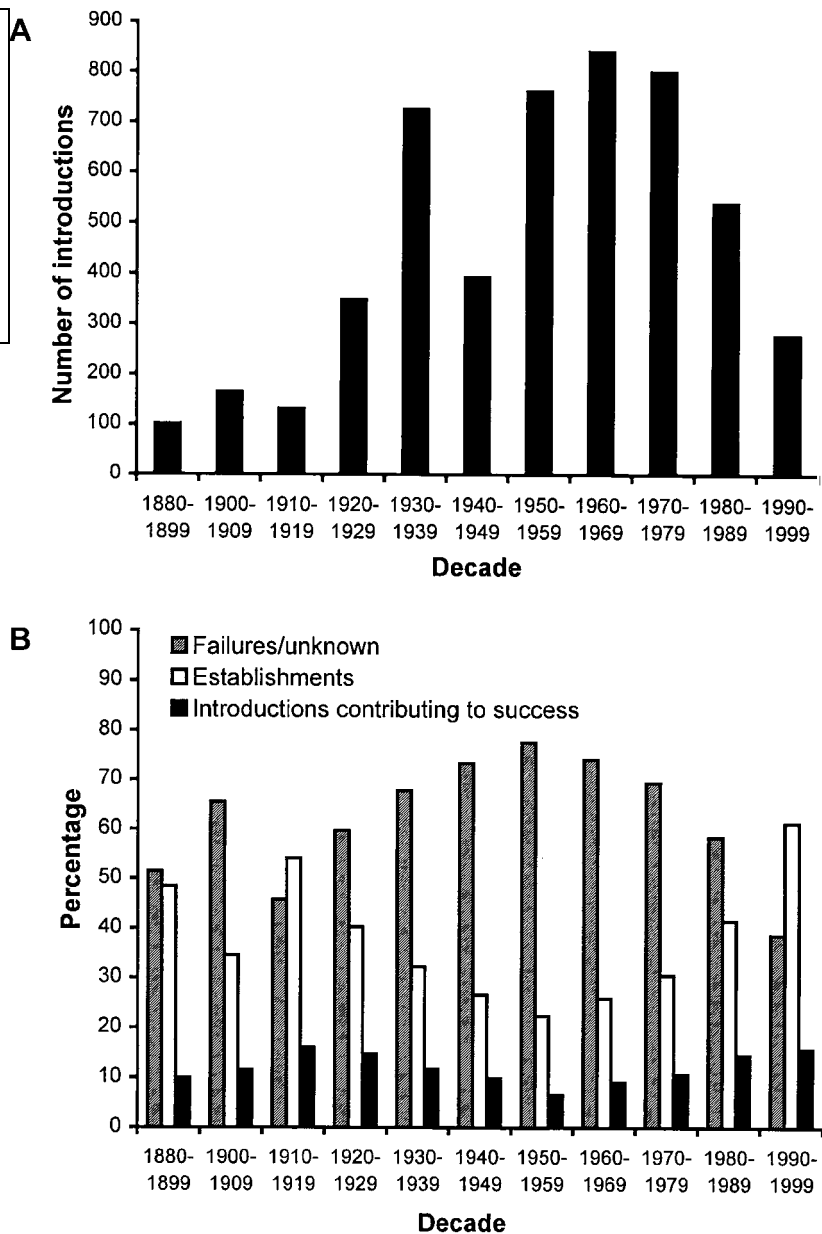
This example provided an early demonstration that biological control could be incredibly effective. This system was, in some ways, unique and had many attributes that foretold success. *R. cardinalis* is very specific, feeding only on scale insects and, even then, its host range is restricted. Most predators are not as specific as the Vedalia beetle. In addition, this beetle had the ability to become established when only a few females were introduced. For example, only four females of this beetle were introduced to Peru to control this same pest and the beetle became established.

against weeds, by 1992 over 340 species of herbivorous invertebrates had been released, also on a worldwide basis.

3.1 | Uses of classical biological control

Classical biological control has predominantly been used for controlling insect pests and weeds and this strategy has been used in very few instances against vertebrate pests (but see Chapter 11) and plant pathogens. The principal types of natural enemies used for classical biological control have been insect parasitoids and predators for controlling insect pests and phytophagous insects for controlling weeds. Some of the most untoward results have been obtained in the past using vertebrate natural enemies, and vertebrates are used only in a few very specific circumstances today (Chapters 7 and 14). Use of pathogens for control of insects trails far behind, with an estimate in 1987 of fewer than 50 programs, across all types of pathogens (Fuxa, 1987). In recent years, obligate pathogens attacking weeds have been successfully introduced in some cases but the number of programs thus far is once again relatively few (see Chapter 15). Avoiding use of microbes in classical biological control has probably been due in part to the increased testing required for microbes by many governments (Waage, 1995). In addition, especially in the early years, it was more difficult to find, identify, and work with virulent microbial natural

Fig. 3.2 Statistics on the results of classical biological control introductions of predators and parasites to control insect pests. A. Numbers of new introductions by decade. B. Percentages of introductions contributing to success (black), establishments (white) and failures or unknown (gray) by decade. (Updated from Greathead & Greathead, 1992.)



enemies for release, compared with macroscopic insect natural enemies. Natural enemies generally considered appropriate for classical biological control are host specific to some extent so that natural enemy populations would increase when hosts increase and decrease when hosts decrease (in a density-dependent relationship; see Chapter 6). Some microorganisms that are obligate pathogens have such relations with hosts, and these seem more appropriate for classical biological control. Why is classical biological control seldom used against plant pathogens? The majority of microbes used for biological

Table 3.1 Statistics on classical biological control of insect pests and weeds using arthropod natural enemies

	Insect pests	Weeds
No. attempted introductions	5576	806
No. of establishments	1866	536
No. of pest species	594	133
No. of agent species	2188	337
No. of countries/islands	239	75
No. of successful controls ¹	625	215

Data through 2001 on insect pests from D. Greathead and on weeds from B. Blossey. For weeds, data after 1996 are not included due to the lag before programs can be accurately evaluated. Data are compiled by country and year so that numerous introductions within the same country are only counted once.

¹ Successful controls include both complete control and substantial control (see Table 3.2).

control of plant pathogens are often ubiquitous microorganisms whose activity is not host specific and whose presence is more often related to the habitat than to presence of the plant pathogen. In addition, these natural enemies are often thought to occur worldwide; there is then no need to reunite introduced plant pathogens with their natural enemies.

Classical biological control programs are considered especially well suited to certain types of systems. Because the goal is to establish natural enemies permanently in a new environment where they will persist, this strategy has been applied more successfully to more permanent ecosystems, such as forests, natural areas, orchards, and perennial crops. This strategy has been used less frequently in short-term agricultural crops. Classical biological control has often been used against pests introduced to relatively isolated areas, such as Australia and islands such as Hawaii or the Californian agricultural area that is isolated by mountains and desert from other North American agricultural areas. For islands in particular, it has been hypothesized that since the fauna on islands is known to be less complex, introduced agents would have better chances of successful colonization. However, summaries of results show that natural enemies released on islands have not established more successfully than on continents, although for those natural enemies that become established, success in control efforts might be slightly greater on islands (Greathead & Greathead, 1992).

Many different types of pests have been targeted by classical biological control programs but there are certain groups that seem to be controlled more successfully. Arthropod pests that are exposed and not hidden and are less mobile have been more successfully controlled because natural enemies have easier access to the pest. For this reason, use of parasitoids and predators against phloem-feeding insects

such as aphids, scale insects, mealybugs, etc. has been very successful, in part because these hosts are fairly sessile and feed externally on plants. This is also the order of insect pest that has been targeted most frequently, in part due to the ease with which these small insects are accidentally introduced to new locations and become pests. This does not mean that important successes have not been made in controlling other groups of pests. Notably, there have been many successful releases against caterpillars, beetles, and flies (Greathead & Greathead, 1992). Perhaps insect pests that live in concealed places have been more difficult targets because host ranges of their natural enemies tend to be ecologically determined and are not always based on the taxonomy of the host. For example, some parasitoids of wood borers are known to attack larvae of long-horned beetles and death watch beetles as well as larval wood-boring bees. However, a narrow host range is desirable so that non-targets will not be affected and host mortality will be density dependent, resulting in regulation of the pest population. Therefore, trying to find natural enemies with a high degree of host specificity for pests in concealed locations can be problematic.

In classical biological control the natural enemy is often released in small quantities, to result in self-perpetuating permanent control. Historically, such programs have been quite inexpensive to conduct and can result in huge savings. Thus, no self-sustaining profit can be generated for private industries to produce the natural enemies. Therefore, governmental or international funding virtually always supports classical biological programs and programs are carried out by international, national, or academic agencies. Because control is permanent and without cost to individuals, this type of strategy has been considered extremely appropriate for pests affecting resource-poor farmers without the resources to pay for pest control. For example, large classical biological control programs against cassava pests in Africa, with funding from international aid organizations, have benefited subsistence farmers with no cost to them.

3.1.1 New associations

Classical biological control was first developed to control pest species that had been introduced from other areas. Today we call these pests invasive species. However, classical biological control has not only been used in this scenario. In some instances, exotic natural enemies have been introduced against a native pest, creating a new association because the natural enemy and the pest had not originated from the same area. Another type of new association has been used when either the area of origin of the pest cannot be found or effective natural enemies cannot be found associated with a pest in its area of origin. In these cases, researchers have searched for and introduced natural enemies attacking similar hosts in other areas. Once again, these are new associations because the natural enemy and pest did not coevolve.

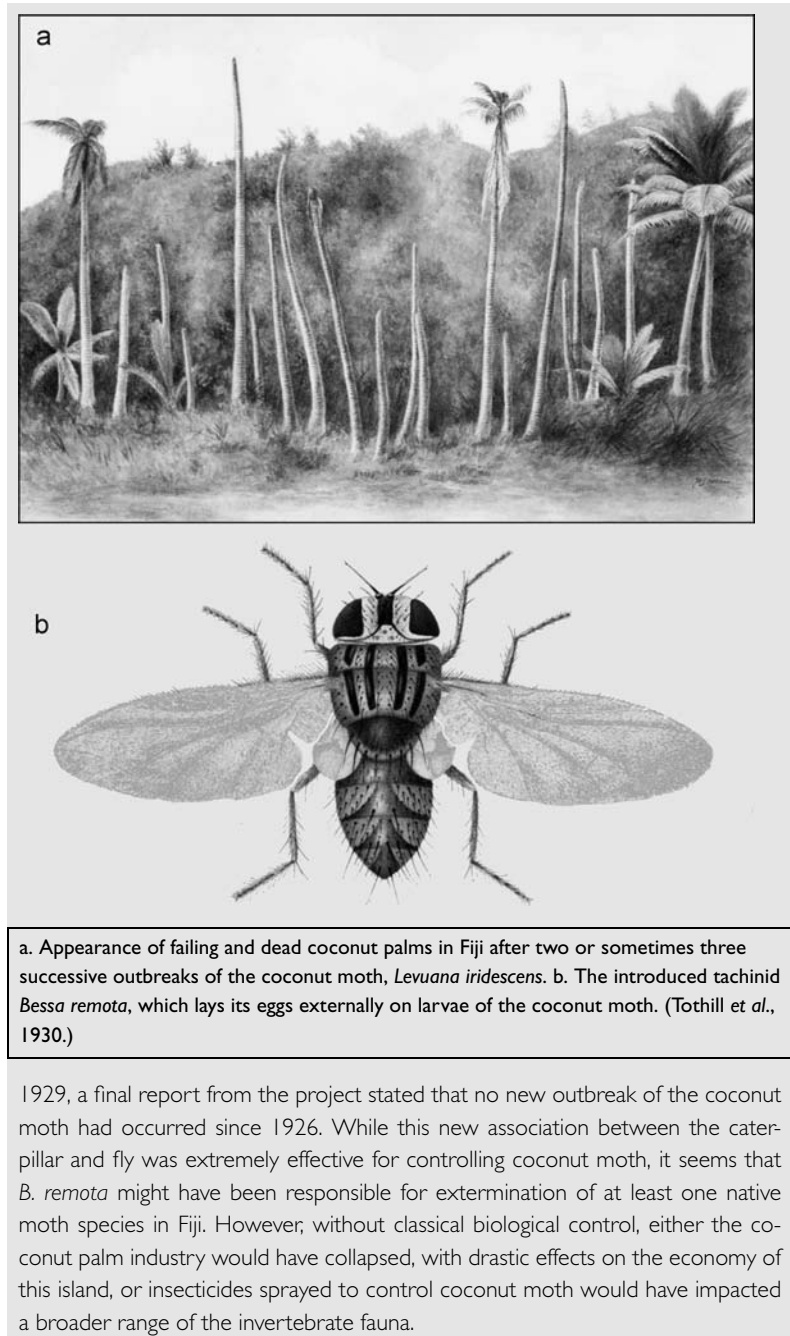
Some scientists have reasoned that interactions between a natural enemy and a pest originating in the same location would have evolved toward a more benign association. Under this scenario, a natural enemy that had coevolved with its host would not be too virulent or efficacious or it would drive the pest to extinction and no longer be able to live. The general concept is that a natural enemy that has not coevolved with a pest could be much more virulent and thus a more effective agent for control.

New associations have not been used as often as coevolved associations, although a review of the literature (Hokkanen & Pimentel, 1984, 1986) demonstrated that they were used more frequently than had been assumed and with greater success. Some uses of new associations have been extremely successful, such as the classic story of the coconut moth in Fiji (Box 3.2). The downside in the use of new associations is that for natural enemies to be successful when they are used in this way, a natural enemy must be less selective in the breadth of host species it will attack or it would not accept a “new association” host that it has never encountered before. While this has led to successful use of new associations, it also suggests that care must be taken to avoid potential non-target effects, a serious consideration for classical biological control programs today (see Chapter 18).

Box 3.2 | Controlling the coconut moth in Fiji

The coconut moth was a devastating pest in Fiji in the 1920s. It was known to have been introduced in 1900 but no-one knew where it had been introduced from. It was thought that the coconut moth might have originated from the larger island Viti Levu, where coconut could not be grown because of the moth. However, when Viti Levu was searched, no natural enemies were found. (Actually, since this early program, our thinking has changed and researchers would now assume that if a pest was out of control in an area, that was probably not where it originated because in its area of endemism, the natural enemies would keep the pest population densities low.) Further South Pacific islands were searched but the coconut moth was not found on any other island.

Eventually, the frustrated researchers decided to try parasitoids and predators attacking related moth species from throughout the southeastern Pacific. At an outbreak of the related moth species *Cathartona catoxantha* near Kuala Lumpur in 1925, both wasp and fly parasitoids were found attacking coconut moth caterpillars. Four large cages were constructed and filled with 85 young palm trees hosting 20,000 parasitized and unparasitized moth larvae. These cages traveled by rail to Singapore and then were on board a ship headed for Fiji for 25 more days. By the time the cages were opened in the quarantine in Fiji, no wasps had survived but a total of 315 individuals of the parasitic fly *Bessa remota* were still alive. These tachinid flies were easy to rear on caterpillars and by 1926, 15,000 flies had been released in coconut-palm-growing areas. These flies did a great job of controlling coconut moth and even proceeded to disperse throughout Fiji on their own. In



3.2 Success in classical biological control

It is difficult to summarize success across the diversity of introduction programs but experience has shown that the percentage of species that are released and provide substantial control is low. To evaluate

Table 3.2 Terminology specific to classical biological control programs

Establishment	Permanent occurrence of an imported natural enemy in a new environment.
Complete control	When no other control method is required or used, at least where the agent is established.
Substantial control	Other control methods are needed but the efforts required have been reduced due to the activity of the natural enemy.
Partial control	While the natural enemy has some effect, other means of control are still necessary (also called “negligible” control).

van den Bosch *et al.*, 1982; McFadyen, 1998.

classical biological control, a set of terms with specific meanings, such as establishment, substantial control, and complete control are used (Table 3.2). Only 33.5% of parasitoids and predators released against insects become established and 66.5% of herbivores released against weeds become established. It is more difficult to evaluate the percentages of releases that result in various degrees of control because scientists vary in their summarizations of results, especially if evaluations after releases were only subjective. Of those programs where a predator or parasitoid became established to control insects, 33.5% yielded complete or substantial control of the pest. Thus, only 11.2% of the attempted introductions of parasitoids and predators against insect pests resulted in complete control (Table 3.1). Somewhat surprisingly, this success rate has not changed appreciably through time (Fig. 3.2B). For arthropod agents established to control weeds, 40.1% yielded complete or substantial control. Thus, of the total attempted introductions against weeds, 26.7% were successful in controlling pests. Why the disparity between success rates for agents to control arthropods versus weeds? Historically, for biological control of weeds, each species for potential release was investigated extensively to determine the host range (see Chapter 18) so perhaps with this increased scrutiny, less promising agents were recognized as such and not released (Gurr *et al.*, 2000a).

Success rates are not greater because results from releases are often unpredictable. Although there are methods that are followed to try to achieve complete control through classical biological control programs, there are many unknowns, especially if working with a poorly understood pest or natural enemy. van Lenteren (1980) very eloquently stated that many decisions while working on a classical biological control project seem more like art than science, often relying on subjective intuition of researchers because objective information about the system is not available. All of the interactions in the environment that could affect the success of a natural enemy cannot always be known before a release and even exhaustive laboratory studies of agents to be released do not always help us to predict the

outcome of releases once the natural enemies are confronted with the pest under field conditions.

How do we improve the success rate of classical biological control? In recent years, the thousands of classical biological control releases have been analyzed by scientists to discover trends in factors associated with successes and failures, ultimately to improve the success rate. Such analyses have identified numerous factors associated with success at different stages during programs. Unfortunately, analyses of classical biological control of arthropods virtually never include weeds, and vice versa. Here, however, we will merge the findings from these two distinct types of classical biological control programs (arthropods and weeds), especially concerning factors that are similar for both, to derive an idea of what factors are associated with a successful program.

3.2.1 Success in establishing the natural enemy

The first step is establishing the natural enemy in the release area. For parasitoids, one study demonstrated that establishment is improved if the climatic adaptations of the host and natural enemy are similar (Stiling, 1990). Yet, so-called climate matching is not always the answer. A study of 178 projects with parasitoids and predators demonstrated that if a species of natural enemy did not become established after the first release, there was a greater chance of successful establishment if a different species altogether was released next instead of releasing further strains of the first species (Clarke & Walter, 1995). For phytophagous natural enemies, faster population growth rate was associated with successful establishment and establishment was improved when more individuals were released or when there were multiple smaller releases (Lawton, 1990). For phytophagous species laying eggs in batches, there was a higher risk of mortality due to predators or parasitoids, and species laying their eggs singly had a greater chance of establishment. Fortunately, it is considered extremely rare for a natural enemy released for classical biological control not to persist after becoming established (Waage, 1990; R. Fuester, pers. commun.). Therefore, although efforts may need to be repeated to establish a species of natural enemy, once that agent becomes established, it rarely goes extinct as long as habitat and hosts are present.

3.2.2 Habitats and hosts associated with success

Classical biological control programs targeting pests in more stable habitats, such as orchards and forests, have been more successful because natural enemy populations persist and are therefore better able to respond to increases in pest density. Often, success occurs in systems that are simpler, as with an introduced natural enemy in a managed system that lacks a complex food web associated with the pest. Also, with a simpler food web, introduced natural enemies might escape the types of enemies that attack them in their native area, such as parasites attacking parasites or predators attacking predators.

Table 3.3 Comparison of use of parasitoids versus predators for classical biological control.¹

	No. of introductions	No. species	No. successes	No. successful species
Parasitoids	4046	1619	468 (11.6%)	238 (14.7%)
Predators	1347	572	122 (9.1%)	48 (8.4%)

¹ Predators are only beetles (Coleoptera) and parasitoids are predominantly wasps (Hymenoptera) and flies (Diptera). Other orders were not included in this summarization, but these were rarely used.

D. Greathead, pers. commun.

Biological attributes of hosts can yield clues regarding probabilities of success. Biological control with parasitoids and predators is more successful against more specialized, instead of generalist, pests. Success is also greater when pests live in exposed locations, such as external feeders on plant leaves versus stem borers (Gross, 1991). Analyses have been extended to evaluate specific groups of pests and one such study looked at the data from 150 biological control programs against caterpillars. Successful parasitoids were most highly associated with two types of hosts: (1) hosts whose larvae were gregarious so that when a group of hosts was located, many parasitoid progeny could be produced, and (2) hosts that were plant feeders specializing on only a few host plant species, perhaps so that natural enemies could easily locate hosts. Predators that were successful were often those attacking smooth-bodied caterpillars and caterpillars protectively colored to blend in with their surroundings (Dyer & Gentry, 1999). For biological control of weeds, there have been more successes in controlling plants with asexual reproduction rather than sexual because the plants are then less diverse and the phytophagous natural enemies can specialize more easily (see Chapter 14 for additional weed attributes associated with success).

3.2.3 Successful natural enemies

There have been numerous lists of attributes of natural enemies associated with successful classical biological control. Successful parasitoids and predators often display (1) good searching ability, (2) a high degree of host specificity leading to a density-dependent relationship with the host, and (3) high fecundity (DeBach & Rosen, 1991). As suggested relative to establishment, the similarity between the climate where parasitoids originated and the climate of the release site can strongly influence whether the pest is controlled (Stiling, 1993). This was shown to be important with the parasitoid *Trioxys pallidus* released against the walnut aphid in California (see Box 3.3). In addition, for parasitoids (1) lack of predators and parasites attacking them in the native fauna and (2) presence of alternate hosts or food have been associated with success. For control of arthropod pests, although parasitoids have been used three times as often as predators (Table 3.3),

Box 3.3 | Some like it hot

The walnut aphid is native to the old world but is now found wherever Persian walnut trees are grown. This aphid was first seen in California in 1911 but only became a pest after 1945. At this time, DDT began to be used to control codling moth on walnuts and it killed other insects, including the predators that had previously kept walnut aphids under control.

In 1959, the parasitic wasp *Trioxys pallidus* was collected from France and introduced to numerous locations in California (Caltagirone, 1981). It became established in southern California but even after 5–6 years of intensive colonization efforts, involving releases of hundreds of thousands of wasps in dozens of locations, *T. pallidus* failed to establish or persist in the northern two-thirds of California, where the majority of walnuts were grown. Robert van den Bosch hypothesized that the French strain of *T. pallidus* might not be well adapted to the much more severely hot summers plus colder winters in northern California, where walnut aphids still flourished. The central plateau of Iran has a climate similar to the walnut-growing areas of California and sure enough, during foreign exploration *T. pallidus* was found there. When an Iranian strain of *T. pallidus* was collected and released in 1968, it became established and dispersed rapidly throughout California with the result that control of walnut aphids was obtained in less than 2 years. Since the introduction of the Iranian biotype, walnut aphid is no longer a problem in California.



The ichneumonoid *Trioxys pallidus* (Family Aphidiidae) parasitizing the walnut aphid, *Chromaphis juglandicola*. (Photo by Jack Kelly Clark, courtesy University of California Statewide IPM Program.)

This example clearly demonstrates that different strains of a natural enemy can differ in climatic adaptation. Even during the time when this study was being conducted, questions arose regarding whether the Iranian biotype was a different species than the French biotype or not. The two strains were morphologically

identical but there were questions regarding whether they would mate and, of course, they seemed to have different climatic adaptations. This problem would be addressed more easily today, because molecular techniques can readily determine relatedness of different strains and we now understand that “cryptic” species can be morphologically identical yet distinct (Clarke & Walter, 1995). An analysis of classical biological control has demonstrated that often when researchers assumed they were working with biotypes, in actuality they were working with cryptic species. There are numerous examples of programs repeating introductions of the “same” species but collected from different areas (so strains are thought to have climatic adaptation). Unfortunately, many such programs have not been able to repeat the success in finding the correct biotype that was seen with *T. pallidus* and the walnut aphid.

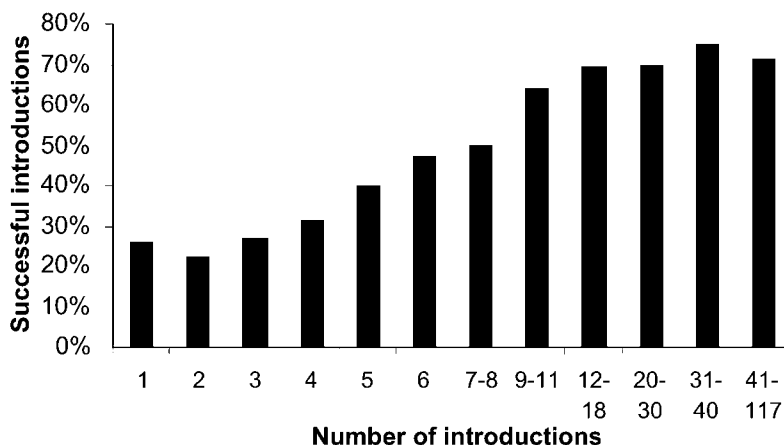
the success rates of these two groups are very similar. The greatest single predictor of success for phytophagous natural enemies of weeds was host specificity; agents that were more host specific were more useful for control (Bergelson & Crawley, 1989).

Pests can be easily moved around the world and then the same classical biological control agents are frequently released in multiple countries; these programs have been referred to as “me too” projects. As you might expect, releasing natural enemy species that were successful elsewhere often leads to success (Lawton, 1990).

3.2.4 Number of releases

As the practice of classical biological control was being expanded, papers written by practitioners questioned how to improve establishment of the natural enemy and control of the pest. There have been heated discussions regarding whether only one or many natural enemies should be introduced; the worry was whether natural enemies might compete with each other and thereby decrease the overall control if numerous species were introduced (see Huffaker *et al.*, 1971). Experience has shown that decreases in control do not occur after releasing progressively more natural enemy species. Today, it is normal practice for numerous species of natural enemies to be released against an arthropod pest or weed due to the unpredictability in results after release. A more recent analysis agrees that as more natural enemies are introduced the probability for success increases, although at least against insect pests this relationship seems to reach a plateau when approximately 9–14 natural enemy species have been released against one pest (Fig. 3.3) (B. Hawkins, pers. commun.). Of course, this means nine or more natural enemy species released for each region; researchers have found over and over again that because an agent is successful in one climatic release area, that does not always mean that it will be successful in another. However, this analysis of the number of species released versus successful control suggests that with increased effort in classical biological control programs there is an increased chance for success; if only one or two agents are

Fig. 3.3 Percentage of successful classical biological control programs using predators and parasitoids against insect pests, associated with varying numbers of species introduced per program. (Data from BIOCAT database, B. Hawkins, pers. commun.)



released, you have much less chance of successful control compared with programs releasing 9–14 natural enemy species.

3.2.5 Length of evaluation affects perception of success

When classical biological control agents are introduced, the effects are often not immediate and patience is necessary before the introduction can be finally evaluated. For parasitoids and predators, approximately 6–10 generations of the pest should occur before evaluation. This could mean less than a year for parasitoids of aphids or scale insects adapted to tropical climates that would have continuous generations all year. However, for host-specific parasitoids of moths in temperate climates that have only one generation each year, this would mean 6–10 years for the same number of generations to occur. For biological control of weeds, an even longer interval has been suggested; it has been suggested that 10–20 years are necessary after the last introductions before program success should be analyzed.

3.2.6 Are expectations realistic?

What of the high failure rate of classical biological control programs? Using natural enemies and pests with characteristics leading to greater chances of success certainly will increase the rate of establishment and successful control. However, we could perhaps also change our expectations about classical biological programs. Although one beauty of some of the examples of complete control has been their low cost, studies have shown that in systems where natural enemies are not successful, increased efforts can improve results. This increased effort can take the form of introducing more agents, within bounds, along with evaluating reasons for failures to gain a better understanding of the system and to try to find a point of weakness or “Achilles heel” of the pest, as a focus for further efforts. The low success rates also are, in part, a perception issue. When we

calculate success rates using the proper lag times suggested above, we find greater success. This can be seen with programs in New Zealand for biological control of environmental weeds that have a success rate of 83% (Fowler *et al.*, 2000).

McFadyen (1998) made the point that successes should not be evaluated by the individual natural enemy species that were released as is usually done but, rather, according to whether a program against a specific pest was successful or not. She suggested we should be asking whether a weed being targeted was controlled, no matter how many agents were released or which agent(s) controlled it. Looking at results in this way, in South Africa, 6 weeds out of 23 targeted were under complete control and 13 more were under substantial control for a total success rate of 83%. In Hawaii, 7 weeds of 21 are under complete control with 3 more under substantial control for a success rate of 50%. Partial (= negligible) successes fall somewhere in between success and failure but are seldom considered in analyses of successes although the natural enemies released are often helping to control the weed to some extent.

Some practitioners of classical biological control feel that the expectations for classical biological control are unrealistically high (Hoffmann, 1995). Historically, programs are considered failures unless permanent, complete control is instituted. Practitioners of biological control of weeds state that natural enemies that have been released but only cause moderate damage to the target weed are still valuable and should be considered as part of integrated control programs. As an example where such an intermediate effect was put to good use, in South Africa, leguminous mesquite trees (*Prosopis spp.*) were purposefully introduced for shade, firewood, and timber in desert areas but then began to invade rangelands. Spread of mesquite was enhanced because livestock love to eat the seed pods and they then spread the undigested seeds so that mesquite began taking over as a rangeland weed. There was a conflict of interest among farmers who used the trees to provide food for livestock and those who did not and wanted the rangeland back. A compromise was reached so that the spread of mesquite could be controlled but the established trees would not be affected. This was done by releasing a natural enemy that only affected reproduction of the trees, a bruchid beetle (*Algarobius prosopis*) with larval stages feeding on seeds. Although this beetle showed great promise, at first control was not apparent because the seed pods were still being devoured by livestock before they could be invaded by beetles. With this knowledge, farmers protected the pods within fenced areas while the beetles were laying eggs and larvae were developing, but they could still feed the pods to livestock after the beetles had emerged. Using this process, the invasiveness of mesquite has been curtailed by the beetles stopping mesquite reproduction and thus arresting further spread of these weedy trees in rangeland, yet established trees are still present in areas where they are wanted (Hoffmann, 1995).

3.3 Economics of classical biological control

Although classical biological control programs cannot always be depended upon to successfully control a pest, they are still widely used because they require a relatively low investment and, with success, control is permanent. Profits are often not apparent for a long time after establishment of the natural enemy, but with success, profits can far outweigh the costs of most classical biological control programs.

Costs for a classical biological control program include finding the natural enemies, identifying them, collecting them, and releasing them. Often few individuals are released, sometimes because they are difficult to rear, yet it is possible to release few individuals because they are expected to increase on their own in relation to the host population and then provide permanent control. Therefore, in general, costs have often been low over the short term for successful programs and, after release, yearly costs of pest control are obliterated.

Cost : benefit ratios from classical biological control programs have been calculated for far too few successful programs, but the benefits always far outweigh the costs and often by considerable amounts. In Australia, an average cost : benefit ratio of 1 : 10.6 was developed by averaging several programs (Tisdell, 1990). This means that for every one dollar used for the program, 10.6 dollars are saved because the crop was not lost and other controls did not have to be utilized. Frequently, cost : benefit ratios from individual programs are much higher, often exceeding 1 : 100. Benefits have been recorded from a program releasing a parasitoid against cassava mealybug in Africa, yielding a cost : benefit ratio of 1 : 200 (Schaab, 1996) and releases of virus against the rhinoceros beetle (*Oryctes rhinoceros*) attacking palms in east Asia and Oceania yielded a cost : benefit ratio of 1 : 120 (C. Lomer, pers. commun.). In fact, all of these cost : benefit estimates are based on some limited interval for the period over which control takes place and money is saved; so, in calculating the cost : benefit ratio for introduction of the *Vedalia* beetle, normally benefits would be calculated for only 10 years after the success in 1890. However, today we are still reaping benefits from the activity of the *Vedalia* beetle so, in fact, the cost : benefit ratio should instead be calculated based on the savings between 1890 and today for a more accurate figure. Of course, cost : benefit analyses only portray the economic benefits and cannot indicate benefits to human health and welfare due to classical biological control successes.

3.4 Methods for practicing classical biological control

Methods for conducting classical biological control programs are relatively straightforward but not especially simple and require several stages (Table 3.4).

Table 3.4 Steps for a classical biological control program against an introduced pest

1. Choose a target pest for which classical biological control would be appropriate and identify its origin. Increasing numbers of countries require that permission for foreign exploration be formally requested.
2. Acquire natural enemies, often through foreign exploration. The natural enemies must be sent to a quarantine to make certain they are without their own parasites or contaminants and for further evaluation.
3. Natural enemies for release will be chosen based on efficacy and safety testing in quarantine. Governmental approval for releases should be sought.
4. The natural enemy will be released in suitable habitats, using best estimates for how many individuals to release and how best to release them.
5. After establishment, distribution of the natural enemy throughout the distribution of the pest is frequently required, especially when the natural enemy does not spread quickly on its own.
6. Evaluation of the activity of the natural enemy. This step can sometimes take numerous years because establishment and increase of the natural enemy is not always immediate.

3.4.1 Determine the area of origin and identity of the pest

For releases against introduced pests, first the origin of the pest must be determined. This sounds easy but has proven difficult in many situations, often because the pest is extremely widespread or the pest is not an outbreak species in its area of origin and therefore is not well known. In the case of the coffee leafminer (*Leucoptera coffeella*), a serious pest of coffee in the New World, this pest and crop have been moved around the world so extensively that the origin of the leafminer can only be guessed. Plants in the genus *Coffea* are native to tropical Africa, Madagascar and neighboring islands, and tropical southeast Asia. Parasites introduced from mainland Africa would not accept coffee leafminer. Reunion, an island in the Indian Ocean off the African coast, hosts several native species of coffee; cultivated coffee was brought to this island in 1718 and then shipped to the New World (Green, 1984). Surprisingly, this leafminer is virtually absent on Reunion. Using the type of reasoning that has been used to identify the areas of origin of natural enemies, it is possible that the coffee leafminer is native to Reunion and is under strong natural control there. However, it is also possible that this pest has some other origin.

The taxonomy of groups including introduced pests is frequently poorly understood and the true identity of a pest may not be known until adequate material has been collected and evaluated by specialists. In particular, because the natural enemies preferred for

biological control are extremely host specific, help from systematists at this stage is critical for obtaining an accurate identification of the pest. The pest identity is critical information necessary for finding the correct host-specific natural enemies. In some cases, classical biological control programs have been unsuccessful until taxonomists reevaluated the identities of pests, only then collecting natural enemies that would yield successful control (see Boxes 8.4 and 14.2).

3.4.2 Foreign exploration

A species of natural enemy can occur across a broad distribution and we have learned that natural enemies from different locations can be adapted to the climate in their area of endemism. Such locally adapted strains have been referred to as biotypes or ecotypes. Some classical biological control programs have only attained success once natural enemies were collected from areas similar in climate to the area for introduction (Box 3.3). Today, climate modeling aids classical biological control in helping provide “guesstimates” on optimal regions for natural enemy searches. For native pests, once again more taxonomic knowledge of the pest and natural enemies would help to pinpoint natural enemies attacking closely related hosts or prey that might be effective against the pest in question.

Once the area of origin has been identified, permission for foreign exploration must be sought from the appropriate countries. Historically, this was not required but an increasing number of countries are requiring permits so that if any profits can be made in the future from natural enemies that are found and exported, the country is aware of this and could potentially benefit.

With permission, a foreign expedition would then be undertaken. Foreign exploration can be difficult and time consuming because in the area of origin of the pest, the pest itself can be difficult to find and natural enemies can be at very low densities. As well as collecting the natural enemies, it is important to gain as much information as possible about the pest and its natural enemies in their area of origin. It can become difficult to decide which natural enemies to emphasize for release, but information recorded as to natural enemy prevalence in the area of endemism could help with such decisions. Any natural enemies that are collected must be cared for properly, so that they remain vigorous. They are subsequently sent to a receiving quarantine.

3.4.3 Quarantine

In the quarantine, the natural enemies must be maintained and, hopefully, increased in number. This usually means that quarantine personnel must also grow the pest to propagate the natural enemy. During rearing, any diseases or parasites of the natural enemies should be eliminated. Researchers found out the hard way that this is important. Potential problems were identified early when a parasitoid attacking another parasitoid (a hyperparasitoid *Quaylea whittieri*) was not recognized as such and was introduced for control of citrus black

scale (*Saissetia oleae*), thus decreasing the effectiveness of primary parasitoids that had been introduced (Askew, 1971) although, thankfully, with time this hyperparasitoid essentially disappeared. In addition, in the quarantine, safety testing should be accomplished so that the host range of the natural enemy is understood.

Foreign explorers might send to the quarantine three small parasitoids that look the same but were collected in different areas. One conundrum has been the occurrence of morphologically identical natural enemies that have very different host ranges. Such groups are called species complexes. The gypsy moth fungal pathogen *Entomophaga maimaiga* belongs to just such a species complex having members with different specificities that can only be differentiated using molecular means or bioassays. Whitefly-attacking parasitic wasps that are virtually morphologically identical can differ in host specificity. As molecular techniques are used more extensively, we can more readily tell whether natural enemies collected in different areas differ significantly and should be considered separately. However, bioassays are still the best way to determine efficacy against different hosts.

To maintain the most effective natural enemies, time in the quarantine should be minimized so that the genetic variability in the natural enemy population is maintained. Also, time in quarantine should be limited to avoid selecting for optimal laboratory growth, trying instead to maintain maximal effectiveness of the natural enemy under field conditions. In fact, field studies in the region of origin of a natural enemy are increasingly being used to complement quarantine studies of host range, both to reduce time in quarantine for natural enemies and to provide more realistic host range data (see Chapter 18).

3.4.4 Planning releases

After a decision is made regarding which natural enemies should be released, governmental permits are required before release. The major requirement for such permits is general knowledge of the host range of the natural enemies so that non-target effects are minimized. The Food and Agriculture Organization (FAO) has developed a set of suggested regulations regarding classical biological control releases (see Chapter 18). As stated earlier, classical biological control releases of microorganisms often undergo more scrutiny compared with release of parasitoids, predators, and herbivorous arthropods.

3.4.5 Releasing natural enemies

As with other stages of classical biological control programs, detailed knowledge of both the host and the natural enemy is necessary to optimize releases. No fail-proof system has been developed for the numbers of natural enemies that should be released in order for the natural enemy to become established. Not all natural enemy species are easy to grow in the laboratory and, in fact, some can be especially difficult to increase in numbers in a quarantine. Due to such

difficulties, it is not always possible to release large numbers of individuals. In such an instance, to ensure chances for mating, practitioners usually release many individuals at fewer sites instead of few individuals at many sites.

Certainly, it makes most sense to release natural enemies where there are large, healthy populations of the pest. For parasitoids, predators, and phytophagous insects, adults are usually released because they are ready to reproduce and will be less exposed to predation and other types of mortality before reproducing. Nevertheless, in some programs, parasitized hosts have been successfully released. For natural enemies like parasitoids, when adults are being released, it is also important to release them in areas where there are nectar sources, so that they will be able to feed. Releasing natural enemies can be fine-tuned even further so that they are released under proper weather conditions to promote establishment. For example, adults of tiny parasitic wasps might be released in shady locations at mid-day on a day with little wind. Insect pathogenic fungi could also be released in the shade and in the evening so that when dew occurs overnight, the fungus can take advantage of the higher humidity. Alternative strategies would be to release arthropod hosts that have been parasitized or infected, introduce plants infested with phytophagous natural enemies, or even introduce resting stages of the natural enemy, such as parasitoid pupae.

After releasing a natural enemy that becomes established, if the agent then spreads slowly, classical biological control programs are generally extended to introduce the agent throughout the pest populations. In Australia, redistribution of phytophagous agents following establishment is now being done through community groups interested in remediation of environmental problems. This community involvement helps to achieve a more-systematic redistribution effort with a more-rapid delivery of biological control results for the public (Briese & McLaren, 1997).

The program for control of cassava mealybug faced a real problem with releasing natural enemies because cassava was a subsistence crop grown over a huge area of central Africa where transportation was difficult or nonexistent. To reach isolated areas most efficiently, adult parasitic wasps were placed in small vials that were dropped from airplanes (Herren *et al.*, 1987). Wasps were able to escape from vials once they reached the ground. This strategy worked because the mealybug host populations were high throughout the release area and the parasitoid attacked all of the stages of the host. Predatory mites attacking cassava green mite (*Mononychellus tanajoa*) were released in a similar way, this time in vials with strings attached to them that would catch on crop plants and provide a route for the predators to crawl onto the foliage.

3.4.6 Evaluation of releases

After releases, it is important to document establishment and efficacy of the released organisms. In fact, if agents do not become established,

they may need to be released again. Different types of agents spread at different rates and classical biological control programs often require additional releases in other pest-infested areas to help speed the spread of an agent. Full efficacy may not be apparent for several years in some systems so evaluation must be conducted after sufficient time has passed. Unfortunately, the length of time for documentation does not always fit funding cycles nor is it standard for different systems but it depends in part on the effectiveness, dispersal, and persistence of the natural enemy.

For some classical biological control programs, agents do not survive well over many years and releases must be repeated (Hunter-Fujita *et al.*, 1998). Subsequent releases after the first release in an area would then be considered augmentation (see Chapter 4). Another example of the need for releasing an agent again would be if the natural enemies are killed inadvertently by pesticide applications. However, in the majority of cases, when natural enemies become established and are left alone, they remain in that area providing control of their hosts or prey.

FURTHER READING

- Altieri, M. A. & Nicholls, C. I. Classical biological control in Latin America: past, present, and future. In *Handbook of Biological Control*, ed. T. S. Bellows & T. W. Fisher, pp. 975–991. San Diego, CA: Academic Press, 1999.
- Caltagirone, L. E. Landmark examples in classical biological control. *Annual Review of Entomology*, **26** (1981), 213–232.
- Caltagirone, L. E. & Doult, R. L. The history of the Vedalia beetle importation to California and its impact on the development of biological control. *Annual Review of Entomology*, **34** (1989), 1–16.
- Gilstrap, F. E. Importation biological control in ephemeral crop habitats. *Biological Control*, **10** (1997), 23–29.
- Kauffman, W. C. & Nechols, J. E. (ed.). *Selection Criteria and Ecological Consequences of Importing Natural Enemies*. Lanham, MD: Entomological Society of America, 1992.
- Pschorn-Walcher, H. Biological control of forest insects. *Annual Review of Entomology*, **22** (1977), 1–22.
- Van Driesche, R. G. & Bellows, Jr., T. S. (ed.). *Steps in Classical Arthropod Biological Control*. Lanham, MD: Entomological Society of America, 1993.

Augmentation: inundative and inoculative biological control

The second and third major ways to use biological control, inoculative and inundative biological control, both involve releasing biological control agents without the goal of permanent establishment. Although these two strategies have different goals and ways in which they work, there are strong commonalities and thus they are usually jointly referred to as augmentation. These strategies are used to control pests when natural enemies are absent, when the control due to natural enemies would naturally occur too late to prevent damage, or when natural enemies occur naturally in numbers too low to provide effective control. The term augmentation is used because natural enemies are being augmented, even when they already occur in the release area but are not abundant enough to provide control.

4.1 | Inundative biological control

The use of living organisms to control pests when control is achieved exclusively by the organisms themselves that have been released
(Eilenberg *et al.*, 2001)

This strategy is directed toward rapid control of pests over the short term. In all cases, no reproduction by the natural enemy is expected. Because control is only due to the released individuals, inundative releases would have to be repeated if pest populations increase again after natural enemies are released. In practice, releases are often repeated if pest populations were not all present in a susceptible stage during the previous application, if new pests disperse into the crop, or if the crop is long lived, increasing the length of time it could become infested. The released agents must contact and kill a sufficiently high proportion of the pest population, or by other means reduce the damage level, to provide control. Of course, to achieve sufficient control rapidly, it is important to release a large number of organisms to inundate the pest population. It has been suggested that microbes being released inundatively must be applied at the density that would be present during a disease epizootic or epidemic because

control would be due to the natural enemies that are released and not their progeny.

Inundative control is often used for short-term crops because viable, breeding populations of the natural enemies do not occur in the habitats provided by temporary monocultures. Alternatively, inundative releases are appropriate where damage thresholds are very low and rapid control is required at early stages of pest infestation.

In many ways, the goals and expectations of this strategy are similar to those for use of synthetic chemical pesticides. Perhaps the similarity of inundative biological control with the pesticide paradigm helps account for the popularity of this approach compared with inoculative release. Natural enemies applied inundatively can be referred to as biopesticides (Hall & Menn, 1999). A few of the many examples of macro-beneficials sometimes referred to as biopesticides when applied inundatively are lady beetles to control aphids, the predatory mite *Neoseiulus cucumeris* to control thrips, and beneficial nematodes to control fungus gnats. Microorganisms that are inundatively applied, for control of arthropods, weeds, or plant pathogens, are often referred to as microbial pesticides for microbial control. An example of these would be the bacterial pathogen used to control numerous species of insects, *Bacillus thuringiensis* (see Chapter 10). Inundative release is also the strategy used to apply a fungal pathogen against locusts in Africa (Box 12.2), a viral pathogen against velvetbean caterpillars (*Anticarsia gemmatilis*) in soybeans (Box 11.2) and a fungal pathogen for control of the weedy stranglervine, *Morrenia odorata*, in citrus orchards (Box 15.1). This latter natural enemy can also be called a bioherbicide or, because this is a fungus, a mycoherbicide.

Strengthening the view that microbes for biological control are similar to chemical pesticides, microbes for inundative release are often sold in forms similar to synthetic chemical pesticides, for example formulated as flowable concentrates or wettable powders, and can be applied repeatedly, often with the same spray equipment that could be used to apply chemical pesticides. However, it has been argued that we cannot think of using these so-called biopesticides in the same way as chemical pesticides. These are living organisms and care must be taken to store and transport them so that they remain alive and are released in an appropriate way (Cook, 1993). Due to the large numbers of natural enemies that must be released when using an inundative approach, methods for cost-efficient and successful mass-production, storage, transport, and release are critical for development and use of this strategy.

4.2 | Inoculative biological control

The intentional release of a living organism as a biological control agent with the expectation that it will multiply and control the pest for an extended period, but not that it will do so permanently

(Eilenberg *et al.*, 2001)

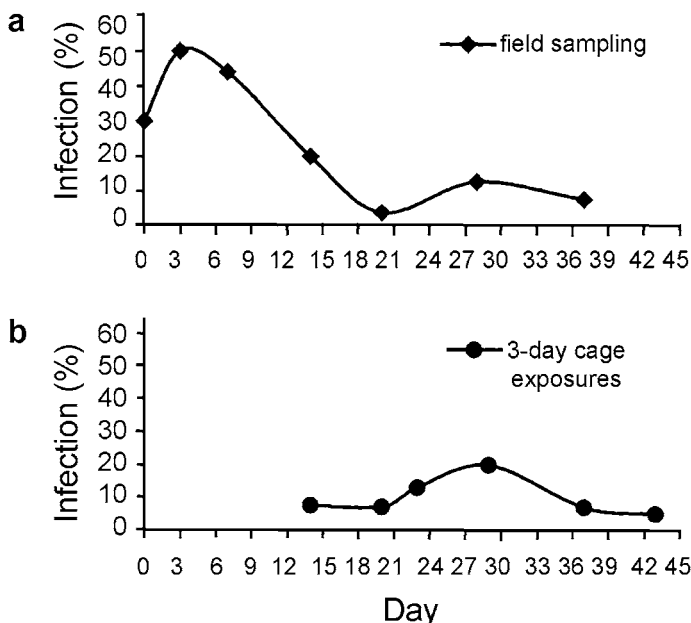


Fig. 4.1 Primary infections initiated after release of *Metarhizium anisopliae* var. *acridum* against African grasshoppers, followed by secondary infections. a. Infection of *Hieroglyphus daganensis* collected at varying times after fungal application on day 0. At each sampling date, 50 grasshoppers were collected and subsequently reared in the laboratory. b. To detect secondary infections, 14 days after the application, healthy grasshoppers were caged in the field for 3-day periods to detect whether infective fungal inoculum was still present in the environment. (Adapted from Lomer *et al.*, 1997.)

For this strategy, control is due not only to the released organisms themselves but also to their progeny. This strategy provides more long-term and self-sustained control than inundative releases. It is used in systems where a natural enemy can respond to and control a pest population, often in a density-dependent manner, but does not persist, or where a natural enemy provides density-dependent control but is difficult to mass-produce in large enough quantities for inundative releases. If an inoculative release is intended for predators, parasitoids, or pathogens, sufficient pest numbers (or other means for growth of the biocontrol agent) must be present following the initial release to support a second or third generation of the released agent, and conditions that allow multiplication of the natural enemy must occur. Studies with the fungal pathogen *Metarhizium anisopliae* var. *acridum* in central Africa have demonstrated this secondary cycling of infection where spores produced from the first cohort of grasshoppers that were killed in the field infect a second cohort (Fig. 4.1). Although fewer natural enemies need to be released than with the inundative approach, these programs still usually require some aspect of mass-production to supply enough agents at appropriate times for release.

For biological control of plant pathogens, in general microorganisms that are released are intended to increase in the microhabitat

where they are released. This is especially true for those antagonists of plant pathogens that are used as bioprotectants so that plant pathogens will not be able to colonize a plant. Clearly, not only the original microbes released but later generations also are needed to colonize roots, wounds, etc. to antagonize disease organisms or protect potential sites of infection.

When persistence in the release area is shortened due to seasonal effects and the natural enemy is released inoculatively each season, this strategy is called seasonal inoculative release. Seasonal inoculative release has been used in greenhouses, where a crop occupies an individual greenhouse for a finite period of time until harvest. Then, the greenhouse is emptied and cleaned in preparation for another crop. The goal of such natural enemy releases in greenhouses is usually to establish populations of natural enemies that will control the pests that are present and then persist to respond to pest upsurges or new invasions while that same crop is present. Under standard greenhouse practices, the natural enemy populations are destroyed during greenhouse clean-out and new beneficials must be introduced into the next crop when new pests are detected. Such use of seasonal inoculative release in commercial greenhouses has been widely practiced in Europe (van Lenteren & Manzaroli, 1999) (Box 4.1).

Seasonal inoculative release is also appropriate to use outdoors for effective natural enemies that cannot persist in an area after release. The egg parasitoid *Pediobius foveolatus* attacking Mexican bean beetle, *Epilachna varivestis*, in soybeans and snap beans has little tolerance to cold weather. Therefore, it has been mass-produced and released each year in mid-spring on a region-wide scale in the mid-Atlantic states in the USA.

4.3 | Inundative versus inoculative strategies

In practice, the distinction between inoculative and inundative releases is not always so precise. An important feature of inundative augmentation is that although the biological control agent is applied without the expectation that it will reproduce, it is a living organism capable of reproduction. In practice, inundative biological control is probably often followed by residual effects if hosts are present and the released organisms can multiply. Conversely, with inoculative release, the majority of control can be caused by the released organisms and the effects from progeny can be minimal, if reproduction is limited.

Whether a natural enemy species is considered for inoculative or inundative biological control is determined in part by the difficulty and cost of producing adequate quantities of that agent for release. For example, while large volumes of the insect-pathogenic bacterium *B. thuringiensis* can be mass-produced in fermenters at a reasonable cost, producing large numbers of parasitic wasps for release is vastly more difficult and costly. In addition, the ability to store,

Box 4.1 | Augmentative releases of macro-biological control agents in greenhouses

In 1970, biological control was used in only 200 ha of greenhouses worldwide while, by 1995, this area had grown to 14,000 hectares out of the total of 300,000 hectares under glass. It has been estimated that more than 80% of the biological control in greenhouses is for cucumber, tomato, and sweet pepper crops. Use for controlling pests on cut flowers (roses, orchids, gerberas, and chrysanthemums) and potted plants (e.g., poinsettias) has not been as common but more recently has been increasing. As a result, pesticide use for control of pests on greenhouse vegetable crops has declined by 85–90%. As of 2000 (van Lenteren), the majority of the use of natural enemies in greenhouses was reported from The Netherlands, the United Kingdom, France, and North America. For example, in Canada, beneficials are used on more than 93% of the greenhouse tomatoes and peppers, and more than 12% of the greenhouse ornamentals (Murphy *et al.*, 2002). To make this increase in use of biological control agents possible, the number of companies producing biological control agents for sale has increased accordingly. In 1968, only two small companies produced natural enemies for sale but the use of biological control in greenhouses in Europe was just beginning. By 2000, 15 companies produced natural enemies in Europe and by 1997, the estimated end-user value of the market for greenhouse biological control agents was more than US\$30 million.



The mite *Phytoseiulus persimilis* (Family Phytoseiidae), an excellent predator of spider mites. (Courtesy of David Evans Walter.)

The most important natural enemies used in greenhouses are the predatory mites *Phytoseiulus persimilis* for use against phytophagous mites, *Neoseiulus cucumeris* against thrips and the parasitic wasp *Encarsia formosa* for use against whiteflies. Due to the fragility of these very small agents, specialized methods for release have been developed. For the winged *E. formosa*, parasitized hosts are glued

onto cards that are then placed throughout greenhouses (Fig. 4.2). When adult *E. formosa* emerge from within the parasitized whitefly hosts, they are ready to fly and find hosts in which to lay their own eggs. Mites are released by sprinkling them on pest-infested foliage. As an alternative, instead of manipulating the parasitoids or predators for release, banker plants inhabited by the host insect and its natural enemies are placed in the greenhouse. Once the natural enemies on the banker plant become overcrowded, they disperse into the greenhouse crop.

Greenhouses are usually scouted to monitor presence of pests and densities of both pests and beneficials. Parasitoids and predators may be released when pests have been seen or they may be released preventatively, based on past history of when pests first occur. For seasonal inoculative releases, determining release ratios can be critical. If too few natural enemies are released, control will not be obtained in time to protect the crop. If too many natural enemies are released, they could drive the host population to extinction so that the natural enemy will also be exterminated and there will be no protection against reinvasion of that crop. Preventative releases, also called blind releases, have become very popular. These are especially used for pests that are difficult to find when scouting but which can increase very quickly, such as thrips that hide within plant parts. Pest explosions must be prevented but during times of high demand it can be difficult to obtain beneficials quickly enough for inoculative control to be effective. For example, in the middle of a growing season for a crop, when many growers need the same beneficials, providers of beneficials can be flooded with unanticipated orders. In such cases, it may take 10 or more days to receive beneficials after the first pests are detected and this might not be acceptable for preventing damage. To solve this problem, natural enemies are ordered regularly and are released on a regular schedule so that they are constantly present in case a pest is introduced or begins to increase. The availability and ease of application of the most commonly used macro-beneficials make the preventative approach simple to use for many pests. While both seasonal inoculative releases and preventative releases can be effective, the former requires more attention and knowledge of the system, while the latter provides excellent protection but more beneficials must be ordered.

transport, and release a certain natural enemy species will help determine whether it can be developed for inundative versus inoculative application.

The ability of those organisms being applied to reproduce after application and for their offspring subsequently to attack hosts influences which augmentative strategy is appropriate. For example, when the insect-pathogenic bacterium *Bacillus thuringiensis* is sprayed, although more bacterial cells are produced in infected insects after they die, any subsequent bacterial generations virtually never go on to infect more hosts. Therefore, use of *B. thuringiensis* is never considered an inoculative application. Conversely, parasitoids are adept at searching for hosts and the progeny of initially introduced parasitoids can have a significant impact on host populations. Therefore, whether parasitoids are intended for inundative or inoculative release, their progeny frequently continue to parasitize hosts, providing the benefits of an inoculative release.

4.4 Production of natural enemies by industry

Use of an inundative release strategy, in particular, requires an industry to produce and distribute natural enemies. In general, companies producing natural enemies specialize in either macroorganisms or microorganisms due to the different types of equipment, methodology, and expertise needed. For both types of natural enemies, although some companies have been in existence for a long time, in this growing field many companies have not been in this business for long.

4.4.1 The need for a market

Augmentative biological control is not seriously adopted by growers unless a steady, reliable product is available to them, while for industry to invest the effort to develop such a natural enemy product, there must be reliable customers that will regularly purchase the product. It is more difficult to develop natural enemies as a product than chemical pesticides because natural enemies often cannot be stored for very long when they are not needed; if not needed and the natural enemies do not survive, the producer or distributor takes a loss. Unfortunately, the need to control pests is volatile and not easy to predict, which can present difficulties for companies producing most beneficials. For a product to be developed, it is critical that the actual market is large enough to support production of that beneficial. Are there customers who will reliably purchase the natural enemy? In general, growers must be educated about biologicals and for the growers to use a biological, it must usually be simple to use and have some significant advantage over pesticides. Examples of such advantages are increased yields when pesticide phytotoxicity no longer causes blossom drop in greenhouse vegetables, increased yields with the use of bumblebees for efficient pollination in greenhouse vegetables (and the supporting avoidance of pesticides so that the pollinators survive), and preference for avoiding use of synthetic chemical pesticides by those exposed to sprays or those purchasing the end-product.

4.4.2 The double-edged sword: host specificity

Host specificity of a natural enemy is often critical to its development for augmentation. Natural enemies with limited host ranges are considered safer for the environment. Also, with a limited host range the natural enemy should respond more closely to increases and decreases in host population density, searching harder when hosts are scarce instead of switching to another host species. Such a density-dependent response is, of course, a more critical feature for inoculative rather than inundative releases because reproduction of the natural enemies released is not expected with inundative releases.

However, host specificity can also influence the size of the market for the mass-produced natural enemy and thus, the final cost of the

Table 4.1 | Steps necessary for developing a natural enemy for augmentative release

1. Identification of a market searching for a pest-control solution
2. Identification of an efficacious strain of a natural enemy for mass-production, both effective against the target and cost-effective to produce
3. Development of a method for mass-production
4. Development of storage methods
5. Development of methods for transport
6. Development of methods for release and quantities needed for release in different situations

product. If the natural enemy attacks a greater breadth of hosts, sales of that natural enemy may be greater depending upon the number of control alternatives for the pest in question. In contrast, a highly host-specific agent would not always generate enough sales to justify mass-production; there must be a market large enough to support the costs of mass-production. In practice, host-specific natural enemies used for augmentation have often been viable if they fill a pest-control niche in a high-value crop.

4.5 | Products for use

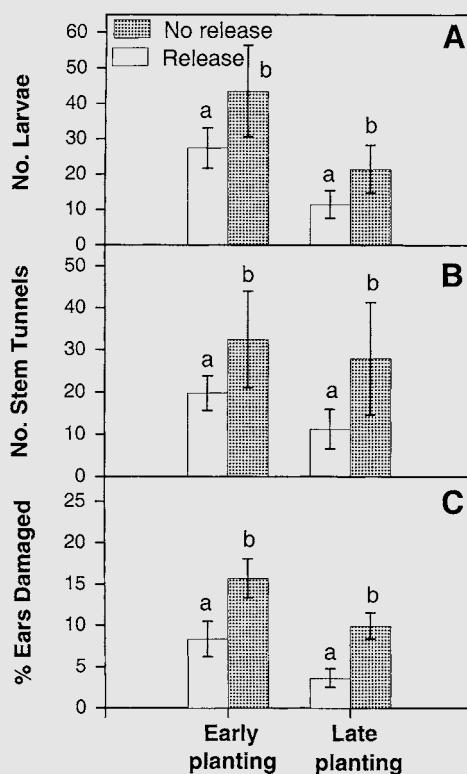
Biological control agents range in complexity from viruses to higher eukaryotes, and methods for mass production, storage, and transport are equally divergent. The species of natural enemies that are chosen for inoculative or inundative releases are chosen in part through trial and error. Often they are known to attack a pest in nature but that is only the start and does not ensure that the species will be appropriate for augmentative biological control. Cost-effective methods for mass-production and handling must be possible for any natural enemy to be mass-produced for augmentative release. For any organism, the process involves several stages, from choosing an efficacious strain for mass-production to developing methods for releasing the natural enemy (Table 4.1). In addition, registration of the natural enemy can be required in some countries, for example, in the USA and Europe for microbials. Because the issues relative to mass-producing and bringing a natural enemy to market are quite different for macroorganisms and microorganisms, these will be described separately.

4.5.1 Macroorganisms

Relative to use for augmentation, arthropod parasitoids and predators are generally referred to as macroorganisms. Although they are very small, they can usually be seen with the naked eye. Parasitoids and predators differ significantly from microorganisms in the ways they can be handled and released and the types of control they provide.

Box 4.2 | European corn borer and *Trichogramma*

The European corn borer, *Ostrinia nubilalis*, is a very difficult insect to control; eggs are laid in clusters externally on corn leaves but as soon as the eggs hatch, the larvae migrate and bore into the corn stem or ear, proceeding to cause damage. This is a difficult pest to control because the larvae live in protected locations most of their lives. Numerous species of the egg parasitoid *Trichogramma* that attack moth eggs have been tested for control of European corn borer using inundative releases. From 194,000 to 484,000 individuals of different species were released per hectare but with variable results. These releases were rarely considered economically feasible in the United States, although results have been different in Quebec and Europe where *Trichogramma* continue to be used against European corn borer.



Mean numbers of European corn borer larvae (A), number of tunnels (B) and percentage ears damaged (C) in *Trichogramma ostrinae*-release and non-release plots of sweet corn at early and late planting dates. Different letters above bars denote statistically significant differences. (Wright *et al.*, 2002.)

Waiting in the wings was *Trichogramma ostrinae*, a species that had not been tested during earlier trials. Mark Wright and colleagues found that this species had quite a capacity for dispersal within fields of sweet corn. These tiny wasps were able to find host eggs after one early season release of 75,000 females/hectare (Wright *et al.*, 2002). This species reproduced and persisted in corn fields throughout the season of release but was unable to overwinter in New York State. Because effects

were due in part to the later generations of the wasps, this is an example of an inoculative release. Releases of such low densities of this parasitoid, without applications of any insecticides, resulted in damage reduction of about 50%. Only one application of *T. ostriniae* is considered necessary and this is estimated as costing about the same per hectare as one insecticide application. While researchers suggest that more work is needed for use of *T. ostriniae* in field corn where success was not as pronounced during field tests, in sweet corn this parasitoid can clearly reduce damage to ears and stalks as well as reducing insecticide use.

Insect parasitic nematodes are generally included with macroorganisms when discussing augmentation. In the USA, parasitoids, predators, and nematodes do not have to be registered with governmental agencies so their development for control is not as difficult as with microbes, which must be registered. Issues for arthropods for biological control of weeds are similar to those for parasitoids and predators although industries for these organisms are very small at present.

Natural enemy strain/species

With parasitoids and predators, biotypes or even species adapted to specific hosts and climates can be extremely important for achieving successful control. In the case of the parasitoid *Trichogramma ostriniae* attacking eggs of the European corn borer, this species has been shown to be so effective that only inoculative releases are needed, whereas inundative releases of other species of *Trichogramma* had repeatedly not been effective enough in the United States (Box 4.2). In fact, augmentative releases of *Trichogramma* against European corn borer in the USA were not considered economically feasible until *T. ostriniae* was found. Biological control researchers are aware that the strain of a natural enemy species can be critically important for efficacy but they are often limited by the strains that are available. Consequently, they may collect new strains worldwide to find unique and useful biological features. Finding new strains is accomplished through exploration but more frequently through obtaining new strains via mail from colleagues in other areas. Of course, when new strains are obtained, efficacy must be tested both in the laboratory and field.

Marjorie Hoy has worked extensively with genetic manipulation of macro-beneficials as a means for improving biological control. For several decades, she and her students worked with laboratory-selected predators to develop strains that were resistant to pesticides used in the field (e.g., Hoy, 1985). In recent years, changing the characteristics of natural enemies has focused on genetic engineering. However, due to the complexity of macroorganisms, to date this technology has not yet progressed far beyond inserting neutral markers in a few beneficial macroorganisms.

Mass-production

While efficacy against the target pest is critical, there will be no product unless the natural enemy can be grown in a cost-effective way.

Macro-natural enemies are mass-produced in a variety of different ways depending on the organisms. However, the vast majority of macroorganisms need living hosts so they must be produced in insectaries where colonies of their hosts/prey can also be grown. The fact that it often requires two arthropod cultures (the host and the natural enemy) to produce one macroorganism product and the resulting product has only a short shelf life helps explain why macro-natural enemies often necessarily cost more than insecticides. Further difficulty is found with cannibalistic predators, such as lacewing larvae, that require considerable space for rearing so that they do not eat each other. In the unique case of the convergent lady beetle, *Hippodamia convergens* (Box 7.4), insects are field-collected and stockpiled, but they become unavailable seasonally and the quality of field-collected individuals is questionable if they are out of synchrony with the seasons at the release site and must be stored for long periods.

One way to make mass-production more cost-effective would be to produce the natural enemies on artificial diets. However, in practice, this technology is used only for producing egg parasitoids in China. Artificial diets are not used more extensively for producing macro-beneficials because methods that have been developed are often not as successful as use of live hosts. There is also concern that natural enemies reared in association with artificial diets will not learn the cues needed to locate hosts or host plants. It is critical not to alter the behaviors of the beneficial that make that species effective for control.

Quality control is an important issue for all agents, no matter how they are reared. There is the potential for inbreeding depression and adaptation to the methods used at the mass-production facility so that the natural enemies will no longer respond appropriately when encountering the pest. The best advice for avoiding such problems is to rear the natural enemy on the target pest on the type of plant or substrate that will be encountered in the field and under normal climate conditions, at least when beginning mass production (van Lenteren, 2000). To ensure quality control, guidelines have been developed by the International Organization for Biological Control (IOBC) for production of the 20 species of macro-natural enemies that are most widely used in greenhouses as well as for the egg parasitoid *Trichogramma* (van Lenteren *et al.*, 2003). To monitor quality over time, population attributes followed include emergence rates, sex ratio, length of the lifespan, fecundity, adult size, and predation/parasitism rate. In addition, a committee of the Association of Natural Bio-Control Producers has developed standards for the quality of natural enemies when these reach the consumer.

Storage and transport

Most macro-natural enemies cannot be stored very long so large numbers must be produced seasonally. Demand for natural enemies is usually not constant so the ability to mass-produce and then store macro-beneficials would be very helpful for maintaining availability. When storage is possible, this allows continuous production of

smaller quantities of natural enemies instead of massive seasonal production. The possibilities for storing macro-beneficials differ by species. For example, diapausing predatory insidious flower bugs (*Orius insidiosus*) can be stored for up to 8 weeks (Ruberson *et al.*, 1998). Methods have been developed for long-term storage of lacewing adults that can subsequently be brought into a reproductive state quickly and synchronously after storage. In contrast, lacewing eggs, the stage that is usually released in the field, can only be stored in the cold for about 3 weeks (Tauber *et al.*, 2000). The entomopathogenic nematode *Steinernema carpocapsae* can be stored for up to 5 months at room temperature but up to a year if refrigerated.

Care must be taken with these living organisms to make sure that they arrive at the release site in excellent condition and are not crushed, asphyxiated, overheated, frozen, or released in transit. It is also often important to maintain humidity within packing containers so that natural enemies do not die of desiccation *en route*. When transit requires several days, food can be packed along with the agents (e.g., honey for parasitoids and pollen or prey for predators). Because predators are often generalists they can be cannibalistic when hungry and at high densities will eat each other with the result that fewer individuals arrive at their destination than were packed initially. Packing cannibalistic species with paper, buckwheat hulls or vermiculite helps to provide hiding places and reduces cannibalism. The excellent courier services available worldwide today make the rapid shipping that is needed very possible; in previous years, shipping services often were not fast enough and macroorganisms that were ordered sometimes arrived in poor condition.

Release

Successful releases rely on a combination of factors that are surprisingly similar to the factors that influence successful pesticide use: application rate, timing (including time of day), synchrony with the pest's susceptible stage, coverage, and severity of rainfall after application. Repeated applications are often needed both for beneficials and for pesticides to assure synchrony of the application with the susceptible stages of the pest. One major difference between biological control and pesticide applications is that beneficial release rates should be adjusted to the density of pests, whereas pesticide application rates are geared to thorough coverage of surface area. Thus natural enemy : pest ratios are more important than active ingredient per acre used for pesticides. Another major difference between releasing beneficials inundatively and applying pesticides is that many beneficial arthropods are still released by hand.

The stage that is released is often determined by ease of transport and manipulation. Depending on the system, releases can be made by hand, as when cards bearing immature stages of the parasitoid *Encarsia formosa*, still within the skins of their dead whitefly pupal hosts, are placed in greenhouses (Fig. 4.2). Predatory mites can be released by dispersing bran containing the mites by hand with a granular-pesticide dispenser or by tractor-mounted applicators.

Fig. 4.2 Card containing whitefly pupae parasitized by *Encarsia formosa* to be hung from plants in greenhouses and interior plantscapes and from which these parasitoids emerge. (Photo by Jack Kelly Clark, courtesy University of California Statewide IPM Program.)



Releases of *Trichogramma* parasitoids in forests and fields have been made by placing cartons containing parasitized hosts on branches or by releasing cartons containing parasitized host eggs from helicopters or airplanes. To avoid predation before emergence of parasitic wasps, host eggs parasitized by *Trichogramma* can be released in fields within capsules that predators cannot penetrate but from which wasps can disperse. Beneficial nematodes are most often applied using conventional pesticide spray equipment.

4.5.2 Microorganisms

Augmentative release is the major strategy used for controlling insects, weeds and plant pathogens with microbial natural enemies. In contrast with macroorganisms, commercial microorganisms are easier to mass produce, store and apply. For industries in many developed countries to sell microbes for augmentation, the microbe must be registered with governmental agencies. Costs of registration can be high so, for an industry to proceed with registration, a microbial product usually must be assured of sustained profits or industries often will not proceed with developing that microbe for control.

Microbial strain/species

Searching for the 'best' strain has been a major occupation of researchers working with microbes for biological control. With microbes, virulence can vary so much from strain to strain that major emphasis is placed on comparing pathogenicity of multiple strains within a species. While virulence studies are always done in the laboratory, plant pathologists strongly suggest that such strain comparisons should also be conducted under field conditions. Especially in the case of soil pathogens, results from the laboratory-bench

can be very different from results in the complex microbial environment of field soil (Whipps & Davies, 2000). For example, the fungal pathogen *Verticillium chlamydosporium* readily infects egg masses of nematodes protruding through galled roots and can provide effective control. However, it was found that different isolates of the fungus had differential survival in the root area, related to the ability of the fungus to colonize the surface of the root. Even different cultivars of tomato affected colonization of the roots by this fungus. Thus, testing microorganisms in the field to determine efficacy can be critical before proceeding further with developing mass-production methods.

The latest development for microorganisms to be used for biological control has been in exploration of manipulations to enhance activity. Many microorganisms have much smaller and more simple genomes than macroorganisms and thus have been targets for use of genetic engineering techniques. Genes have been inserted into viruses, bacteria, and fungi used for biological control to (1) enhance virulence, (2) confer resistance to pesticides, and (3) alter host range. Field trials have been conducted by releasing genetically engineered microbial agents in limited areas, with the first studies only releasing microbes expressing genetic markers. Initially, these field trials raised a distracting media furor but now field studies can be conducted with more focus on results. While few products on the market are genetically engineered, at present engineering microbes for biological control is an active field of research.

Mass production

The microorganisms that have been used the most to date, for example the bacterial pathogen *Bacillus thuringiensis*, require simple media and are relatively cheap to mass-produce in larger fermenters. For other species, it has been important to spend time optimizing nutrients (carbon sources, carbon to nitrogen ratios, etc.) and the fermentation environment (temperature, pH, aeration, etc.). Some microbial natural enemies do not readily or abundantly produce propagules, such as spores, in culture and strain selection as well as growth conditions have been manipulated during attempts to overcome this obstacle. In fact, spore production or the lack of it during mass-production has determined the success or failure of species being considered for development as bioherbicides.

Other microorganisms, such as viruses and some obligate pathogens, can only be grown in living hosts. The need for living hosts can seriously influence the extent to which microbial natural enemies are mass-produced. Among the fungi and bacteria, products are dominated by species and strains that are easy to grow in culture without live hosts. However, just because the natural enemy cannot be mass-produced outside of hosts has not stopped large-scale production of some insect pathogenic viruses (see Chapter 11).

Quality control issues with mass-produced microbes include (1) assurance that cultures have not become contaminated, especially by microbes pathogenic to humans, (2) assurance that cultures are

Fig. 4.3 Using an airplane to spray a microbial natural enemy such as *Bacillus thuringiensis* or nuclear polyhedrosis virus for arthropod control in forests. (Photo courtesy of J. Podgwaite, USDA, Forest Service.)



still virulent to target species, and (3) assurance that active unit numbers, such as fungal spores, are as stated for the product.

Storage and transport

Some microbes can be stored for months or years at room temperature, for example, *B. thuringiensis* spores are thought to survive decades, if not longer. For these species, storage, shelf life, and shipping are similar to synthetic chemical pesticides. This is an important advantage because it allows year-round production and easy storage until the product is needed. Some fungi are more fragile and can be stored only for several months, often with refrigeration. The entomopathogenic fungus *Verticillium lecanii*, sold to control aphids in greenhouses, is viable for a few months when kept cold.

Release

Another advantage of microbes is that they can often be applied with pre-existing equipment used for synthetic chemical pesticides. For materials that can be produced in large quantities and that need to be applied over extensive areas such as large fields, forests, or rangeland, application is possible from the air (Fig. 4.3). In contrast with the macro-natural enemies, microbes must be deposited closer to the correct location of the pest because they have less ability to disperse and locate the pest, compared with mobile parasitoids and predators.

Microbes are almost always mixed with other materials to facilitate application; this is called formulation (see Burges, 1998). Many microbes are sensitive to desiccation and solar radiation and cannot protect themselves after application. Therefore, formulations can include aids for extending the lives of microbes, such as protectants against ultraviolet radiation. Formulations can also include materials added for delivery to the target pest, such as materials that help the microbe stick to a plant leaf instead of directly washing off. Formulations can help improve the activity of a microbe; for example optical brighteners added to insect pathogenic viruses help to compromise

the gut wall of a caterpillar so that the virus can infect more readily. Fungal spores that require free water for germination can be formulated in vegetable oils that retain water; these formulations are then applied using ultra-low volume application equipment. Microbes can also be formulated to improve storage, ease of use, and compatibility with application equipment used for spraying pesticides.

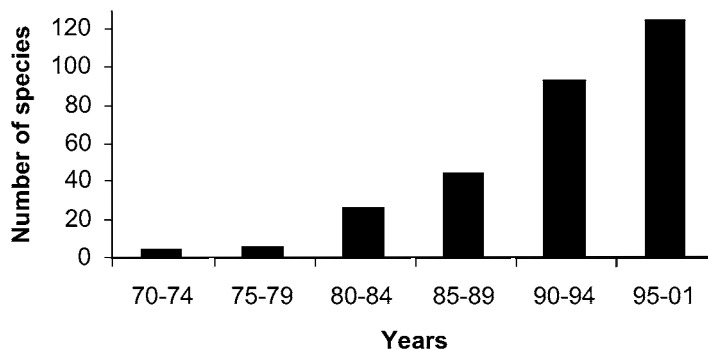
4.6 Regulation

In the United States and Europe, macro-natural enemies and entomopathogenic nematodes are not regulated to the same extent as microorganisms and they do not have to go through extensive safety testing before use. In stark contrast, microorganisms being developed as pest-control products are regulated by a three-tiered testing system, although this is much less rigorous than the testing required for chemical pesticides. The toxicity tests required in the USA have been estimated to cost from \$200,000 to \$500,000, in comparison to the multimillion dollar requirements for chemical pesticide registration. In the UK, the cost of registering a microbial biological control agent is only 25% of that charged for chemical pesticides. However, the country-by-country registration requirements have been cost-prohibitive for the majority of microbials because many of these are for only small markets. Particularly when the biological control agent is targeting a niche market, such as a crop that is not grown over huge areas, registration often simply costs too much to justify industry investment toward developing and producing the beneficial. Antagonists used for control of plant pathogens can sometimes sidestep this problem by being sold as plant growth promoters, soil conditioners, plant strengtheners, or wound protectants, thus avoiding the need for registration and the associated costs of testing (Whipps & Davies, 2000). However, for microbes used to control arthropods or weeds, regulatory requirements in developed countries constitute a great deterrent for industry considering development of microbial biopesticides.

4.7 Natural enemies commercially available for augmentative releases

Macro- and micro-natural enemies are mass-produced for augmentative release by a great diversity of organizations, from large or small companies to farmer's cooperatives and national research organizations. Regional companies facilitate distribution from producers. Current information on natural enemies that are available commercially and where they can be ordered can be found in reviews (Cranshaw *et al.*, 1996, van Lenteren *et al.*, 1997), in directories that are updated annually (e.g., Quarles, 2002) or every few years (Copping, 2001), or on websites such as the website of the Association of Natural Bio-control

Fig. 4.4 Increase in the number of species of commercially available natural enemies for augmentative biological control of greenhouse pests in Europe. (Updated from van Lenteren, 1997.)



Producers (USA) (www.anbp.org). Of course, not all natural enemies are available in all countries and permits are needed for moving natural enemies from one country to another. Suppliers often provide information about quantities to order, timing, and procedures for releasing natural enemies.

In the year 2000, more than 130 species of insect and mite predators and parasitoids were available commercially worldwide (van Lenteren, 2000; van Lenteren, pers. commun.) (Fig. 4.4). About 30 species make up 90% of the total sales. Use of numerous species of antagonists to control plant pathogens is increasing in greenhouses (see 19.4.2). Many of these natural enemies are used in exceptionally successful augmentation programs in greenhouses (Box 4.1). Intensively managed greenhouse crops are especially well suited for augmentative biological control because many available pesticides kill the bumble bees used to pollinate greenhouse vegetables or produce phytotoxic effects in plants, including premature abortion of fruit and flowers. Releasing natural enemies takes less time than spraying chemical pesticides and there is no period after application when workers cannot re-enter (for chemical pesticides there is usually a period after application during which people cannot re-enter the sprayed area) and some key pests can only be controlled with natural enemies (van Lenteren, 2000). Greatly increased yields associated with the use of natural enemies in high-value greenhouse crops have justified their use instead of pesticides for decades. In the past, adoption of augmentative biological control was primarily for greenhouse vegetables, driven by the need to minimize pollinator injury and minimize pesticide use on food crops. Today, use is also common on ornamentals, such as tropical foliage plants, roses, and *Gerbera* daisies.

Augmentative uses of macro-natural enemies in the field have not been as extensive as greenhouse use but natural enemies have found their niches. For example, predatory mites (*Phytoseiulus persimilis*) have been widely used for control of two-spotted spider mites (*Tetranychus urticae*) in strawberry fields in California for more than a decade. The most widespread application of macro-beneficials in the field worldwide may be the use of the hymenopteran egg parasitoid *Trichogramma*. These egg parasitoids are mass-produced around the world to control caterpillars in a variety of ecosystems; for

example, in China *Trichogramma* are applied in cereals, industrial crops such as soybean and sugar cane, vegetables, and fruit and forest trees (van Lenteren, 2000). Insect-attacking nematodes are used against numerous soil-dwelling pests in turf and a diversity of smaller crops.

In addition, phytophagous arthropods, predominantly beetles and caterpillars, are available for augmentative release against weeds. However, these are mostly intended for inoculative release in areas where these species have not yet been released or did not survive.

The principal use of microorganisms in biological control is augmentative release. Approximately 80 products based on bacteria and fungi were available for control of plant pathogens in 2000 (Whipps & Davies, 2000). Numerous products based on bacteria, viruses, and fungi are available for control of arthropod pests. A few species of fungi have been mass-produced for control of specific weeds. However, among all of the microbial products, the most important is *Bacillus thuringiensis*, accounting for the majority of the US\$75 million per year in sales of natural enemies for augmentative biological control (Lisansky, 1997).

FURTHER READING

- Albajes, R., Gullino, M. L., van Lenteren, J. C. & Elad, Y. (ed.). *Integrated Pest and Disease Management in Greenhouse Crops*. Dordrecht, NL: Kluwer Academic Publishers, 1999.
- Burges, H. D. (ed.). *Formulation of Microbial Biopesticides*. Dordrecht, NL: Kluwer Academic Publishers, 1998.
- Copping, L. G. (ed.). *The BioPesticide Manual*, 2nd edn. Farnham, UK: British Crop Protection Council, 2001.
- Evans, H. F. (ed.). *Microbial Insecticides: Novelty or Necessity?* Farnham, UK: British Crop Protection Council, 1997.
- Hall, F. R. & Menn, J. J. (ed.). *Biopesticides: Use and Delivery*. Totowa, NJ: Humana Press, 1999.
- Hoy, M. A. & Herzog, D. C. (ed.). *Biological Control in Agricultural IPM Systems*. Orlando, FL: Academic Press, 1985.
- Paulitz, T. C. & Bélanger, R. R. Biological control in greenhouse systems. *Annual Review of Phytopathology*, **39** (2001), 103–133.
- Ridgway, R. L., Hoffmann, M. P., Inscoc, M. N. & Glenister, C. S. (ed.). *Mass-Reared Natural Enemies: Application, Regulation, and Needs*. Lanham, MD: Entomological Society of America, 1998.
- van Lenteren, J. C. Success in biological control of arthropods by augmentation of natural enemies. In *Biological Control: Measures of Success*, ed. G. Gurr & S. Wratten, pp. 77–103. Dordrecht, NL: Kluwer Academic Publishers, 2000.
- van Lenteren, J. C. (ed.). *Quality Control and Production of Biological Control Agents: Theory and Testing Procedures*. Wallingford, UK: CAB International, 2003.

Conservation and enhancement of natural enemies

This strategy for biological control differs from classical biological control and augmentation because natural enemies are not released. Instead, the resident populations of natural enemies are conserved or enhanced. There is a level of debate in the biological control community regarding what to call this strategy or even how to define it. Here, we use the definition of conservation biological control supported by DeBach (1964b), Barbosa (1998), and Eilenberg *et al.* (2001).

Modification of the environment or existing practices to protect and enhance specific natural enemies or other organisms to reduce the effect of pests

In fact, this strategy was first principally developed to conserve natural enemies that were being decimated through use of synthetic chemical insecticides (van den Bosch & Telford, 1964). Conserving natural enemies only later began to be linked with enhancing them. For many years, our knowledge of how to conserve and enhance natural enemies grew only haltingly. These are more passive approaches and are usually directed toward long-term control of pests. Conservation methods are usually not suitable for control of pests in high value crops that can withstand little damage (have a low economic injury level). A fundamental requirement for using conservation and enhancement is that the biology, behavior, and ecology of the pest and natural enemies must be understood to some extent. To develop effective conservation and enhancement of natural enemies we need to understand what factors are depressing natural enemy populations or otherwise inhibiting their ability to control pests, and these detractors must be alleviated. Alternatively, those factors limiting natural enemy populations must be identified so that they can be manipulated to enhance population levels of natural enemies or facilitate interactions between natural enemies and pests.

In more recent years investigations of conservation and enhancement have blossomed for control of insects as well as plant diseases and plant parasitic nematodes. For arthropod control, a diversity of types of methods has been developed and these will be briefly described in this chapter (Table 5.1). As a type of conservation and

Table 5.1 Diversity in methods used for preserving and increasing natural enemy numbers and activity*Conservation*

Altering pesticide use

Enhancement

Providing food, often nectar and pollen sources

Providing permanent habitats, shelter and favorable microclimate

Providing alternate prey or hosts (often present naturally in more diverse habitats)

enhancement, plant pathologists and nematologists use communities of organisms that develop within suppressive soils to control pests; this is described in Chapter 16.

5.1 Conserving natural enemies: reducing effects of pesticides on natural enemies

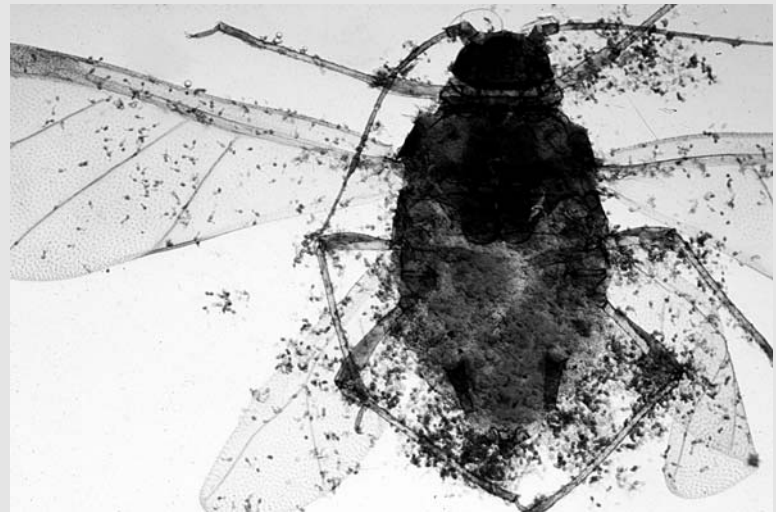
If broad-spectrum pesticides are used as the principle method for controlling a pest, natural enemies are usually disrupted too much to be effective. To utilize resident natural enemies, most growers must change their goals and integrate use of pesticides that do not disrupt natural enemies. Growers can collect data about the status of the pest in the crop system and then follow decision guidelines that provide thresholds when insecticide treatment should begin to avoid economic loss. Thus, unnecessary spraying will not deplete natural enemies. When pesticide applications are necessary, hopefully insecticides can be chosen and applications can be timed to preserve natural enemies. Such a scenario has been shown to be very effective at reducing pesticide inputs in numerous systems.

A specialized program to take advantage of a fungal pathogen of cotton aphids has been successfully used to drastically reduce pesticide use for control of cotton aphids in the southeastern USA for 10 years (Box 5.1). In this case, the pesticide does not kill the natural enemy but growers can take advantage of naturally occurring fungal epizootics instead of spraying to control the pest.

In a worst case scenario, use of agricultural chemicals directly kills natural enemies of the target pest or, by killing natural enemies of other organisms in the environment, secondary pests are created, thus starting the pesticide treadmill (see Chapter 1). Chemical pesticides also often reduce pest numbers to such low levels that natural enemies cannot persist. Because pests are often great dispersers and faster colonizers than natural enemies, the pests recolonize areas more quickly, leading to habitats devoid of predators and parasitoids. Synthetic chemical pesticides do not always kill natural enemies but can decrease their longevity and fecundity, thereby decreasing their

Box 5.1 | Taking advantage of fungal epizootics

Cotton aphids were not considered major pests in the USA until population outbreaks after insecticide applications began for control of boll weevil (*Anthonomus grandis*) in the 1940s. Cotton aphid outbreaks at that time were attributed to elimination of their natural enemies by insecticides. After a hiatus, cotton aphids again became problematic in the 1980s due to development of resistance to pesticides at the same time that broad spectrum pesticides suppressed natural enemies. Aphid populations can increase rapidly and the honeydew produced by their feeding can make cotton lint black, dirty, and sticky, decreasing its value.



Dead cotton aphid, *Aphis gossypii*, on a microscope slide. Growth of the fungal pathogen *Neozygites fresenii* is indicated by fungal spores on the aphid's wings and fungal cells growing throughout the body. (Photo courtesy of Donald Steinkraus.)

In 1988, Don Steinkraus observed that cotton aphids were being killed in great numbers by a fungal pathogen, *Neozygites fresenii*, that had never before been reported from this aphid in cotton. The humid weather during the field season in Arkansas and surrounding states was perfect and this fungus could rapidly spread and decimate cotton aphid populations. This is an obligate pathogen and cannot be grown outside of living hosts, so mass-production of this fungus for application was not an option. Steinkraus took a different approach and decided that if the epizootics caused by this fungus were occurring anyway, growers could take advantage of them. In 1993, he began a service whereby growers detecting abundant aphid populations and considering spraying could submit aphid samples taken in a standardized way. A random sample of 50 aphids was observed under the microscope to detect fungal infections. If the aphid population is large and the fungus is present in approximately 15% of samples or more, then there is a high likelihood that during the 5–7 days after sampling, an epizootic will occur. Therefore, the grower does not need to apply insecticides. If the grower applies pesticides, he would be wasting his money because the fungus would control the

pest. If permanent plant damage is being caused by an aphid population and no fungal infection or only low levels are found, the grower should not expect control by the fungus and should take alternate action to protect the crop.

When this service began in 1993 it was only used in Arkansas. Ten years later it is still being used and has expanded to five southeastern states. This aphid fungus benefits growers by eliminating aphid populations naturally and thereby preventing applications of pesticides that would cost money as well as killing other natural enemies of cotton aphids as well as natural enemies of other pests.

effect on pests. Thankfully, not every natural enemy is killed by each agricultural chemical; some species are tolerant and some have developed resistance. With knowledge of specificity of action, effective chemicals can sometimes be used that will not harm natural enemies; these are called “selective pesticides.”

Pesticides can also be applied so that susceptible natural enemies are not exposed to them. For example, granular formulations that are applied to the soil would not affect natural enemies on the foliage. Systemic pesticides taken up by plants would not affect natural enemies that do not feed on the plant. However, as a warning regarding systemic pesticides, if the pests that are feeding on the plant are not affected by the systemic pesticide and it accumulates in their bodies, natural enemies that are sensitive and then attack pests can be affected.

Treating only part of a crop with a pesticide, for example applications to alternate rows of apple trees, provides a habitat where predatory lady beetles feeding on phytophagous mites can survive (Hull *et al.*, 1983). Altering timing of treatments can also result in reduced exposure of natural enemies. Nonpersistent pesticides can be applied or applications can be made infrequently. Better yet, pesticides could be applied when natural enemies are not present during the season or when they are in a protected stage such as during pupation. For example, models were used to predict development of a parasitoid and its host, the cereal leaf beetle, *Oulema melanopus*, so that pesticides were applied in the spring before parasitoids had emerged (Gage & Haynes, 1975).

5.2 | Enhancing natural enemy populations

Habitats must provide resources needed by natural enemies, such as food, hosts or prey, shelter, and acceptable abiotic conditions. Often crop habitats fail to provide these resources or they are not provided where or when natural enemies occur. To enhance natural enemy populations, we must learn what resources are limiting the natural enemies and devise methods to provide these limiting resources at the correct time and place. Therefore, understanding the biology and ecology of organisms in a system is critical. Due to the variability among species occurring in different systems, methods for natural

enemy enhancement that are effective in one system are often not transferable to other systems.

Almost every enhancement strategy can be seen as some method for manipulating the habitat to increase densities of natural enemy populations or to increase natural enemy effectiveness in controlling pests. As early as AD 900, Chinese citrus growers placed nests of the predaceous ant *Oecophylla smaragdina* in mandarin orange trees and constructed bridges between trees, to enhance dispersal of predatory ants and thus reduce populations of foliage feeding insects. Farmers have known for centuries that some habitats are more amenable to naturally occurring biological control than others and, when possible, have adjusted farming practices to take advantage of this (Pickett & Bugg, 1998).

A great diversity of methods has been investigated for enhancing natural enemy occurrence and effectiveness. However, enhancement due to many of these practices has not been effective enough for adoption or continued use, especially in developed countries. Rabb *et al.* (1976) in a review stated that “Most of the techniques . . . are of potential rather than realized value in pest management.” While this statement was made in 1976, the overall situation has not changed appreciably today (Ehler, 1998). Perhaps in this biological control strategy, more than the others, the gap between information learned by scientists and its use in the field is the greatest. This gap has been called the “valley of death,” where results from research do not become implemented as practice (Office of Technology Assessment; US Congress, 1995). Perhaps the “valley of death” is caused in part by the fact that few truly efficacious strategies have been developed. In some cases, although viable conservation strategies have been developed, at least in developed countries the economics of agriculture and integration with prevailing production practices take precedence, and ecologically sound environmental modifications to enhance natural enemy populations are usually not adopted.

With the publication in 1998 of two books specifically focussing on natural enemy conservation and enhancement (Barbosa, 1998; Pickett & Bugg, 1998), these strategies received renewed interest. In theory, these strategies certainly are well suited to the newer pest management approaches integrating different control methods, such as integrated pest management and sustainable agriculture (see Chapter 19). While enhancement strategies are more difficult to adapt to the large acreages of major crops grown in developed countries, they could be more appropriate for smaller farms in developing countries. Certainly, organic growers and growers working toward decreasing pesticide use are very interested in methods for habitat management that take advantage of resident natural enemies. However, methods must be tailored so they are still affordable because increasing the complexity of manipulations for production and control can imply costs due to increased manual labor and this is often not acceptable to growers.

5.2.1 Theory underlying vegetational diversity and biological control

In conservation biological control, manipulation of vegetational diversity to enhance natural enemies has been a major focus, based in part on the following findings. In 1973, Dick Root planted collards either in pure stands or in single rows surrounded by meadow vegetation. He found fewer herbivores and more natural enemies in the single rows. Based on his findings, he proposed a “resource concentration hypothesis” related to the dynamics of phytophagous arthropods which stated that “herbivores are more likely to find and remain on hosts that are growing in dense or nearly pure stands.” This, of course helps explain associations between large acreages in monoculture (growth of one type of plant) and associated extensive pest problems. Herbivorous pests have no problems at all finding crop plants that are their hosts when crops are grown in large monocultures.

Associated with this same theme of vegetational diversity affecting plant–arthropod relations, based on his results, Root proposed an “enemies hypothesis” stating that natural enemies are more abundant in diverse plant communities. Thus, an extension from greater arthropod diversity in the plant community with the single row planting was a resulting greater pressure on herbivores from natural enemies. The take-home message from this system was that increasing plant diversity was associated with decreased herbivore populations and increased natural enemies. This sounds encouragingly straightforward but, when investigated in other systems, the same patterns are not always found.

While no one rule seems to explain the relations between vegetational diversity and natural enemy abundance, some studies have supported Root’s 1973 proposals. A review of the literature (Coll, 1998) showed that, among studies on field crops comparing monocultures with fields where several plant species were grown in the same field, parasitoids were more abundant in 72% of the cases with diverse plants. One recent study clearly demonstrated that in systems with more vegetational diversity, by providing non-crop areas adjacent to fields of oilseed rape, parasitism of the pestiferous rape pollen beetles, *Meligethes aeneus*, was higher and crop damage was lower (Thies & Tschardtke, 1999).

Along the same lines, numerous studies have shown that, as a general rule, fewer crop pests are found as the diversity of the system increases (Andow, 1991). A review of 209 studies of 287 herbivorous arthropod species found that approximately 52% of the species had lower population densities when vegetation was diverse in fields because several crops were grown, a cropping practice called polyculture, while 15% of the herbivores had higher densities in polycultures. Thus, while arthropod species had about a 50% chance of being less abundant in polycultures, there also could be more species feeding on plants in polycultures than in monocultures. For the cases where herbivore numbers are lower in more diverse habitats, what is causing

this trend? It is not the plant diversity *per se* that leads to lower pest numbers through increased natural enemies, but the resources that diversity provides to the beneficial organisms.

In fact, sometimes vegetational diversity provides needed resources for natural enemies and other times it does not, as shown by the occasional occurrence of higher densities of herbivores in polycultures. In more diverse systems, parasitoids and predators might have more difficulty finding prey and hosts and having a diversity of plants could benefit the herbivore, or diverse systems could support greater populations of the natural enemies that kill the parasitoids and predators. Unfortunately, each system is idiosyncratic and must be considered separately to understand whether specific manipulations hinder or favor pests (Bugg & Pickett, 1998). While it is clear that vegetational diversity can have a profound effect on herbivores and natural enemies, there seems to be no overarching theory that consistently explains the relative importance to pest density of pests and natural enemies in simple versus diverse systems. Once more, these relations seem to be system specific and the trick is to discover what resources are limiting natural enemies and determine how these can be added to the system.

5.2.2 Enhancing habitat for natural enemies: within a crop

Many crops are grown today in simple monocultures which, without change, may not provide the resources required by natural enemies. Food and shelter especially can be minimal after harvest and before a field is replanted. This situation can be altered in various ways, from providing microclimate and shelter to providing alternate food, including nectar, pollen, and alternate hosts or prey. In addition, the crop habitat is often transient so that populations of natural enemies cannot be retained from year to year, much less increase over time. Management of crops can be altered in a great diversity of ways to preserve and enhance natural enemies. However, few of the methods that have been investigated are actually in use, perhaps because few have been shown to result in suppressed pest populations with an adequate decrease in pest damage. Here, some of the diversity of manipulations is described.

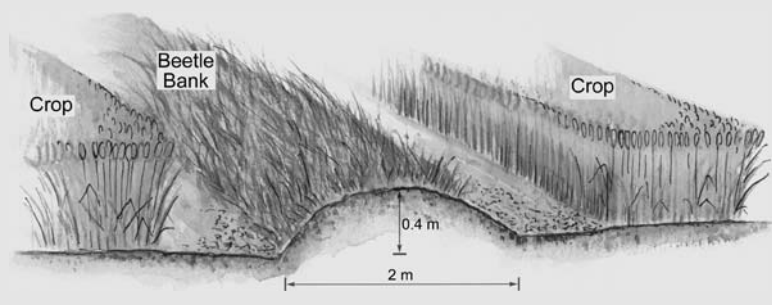
Providing refuges within a crop

Construction of natural areas that occupy limited areas within fields provides an excellent example of very successful control that has been accepted by growers. One of the most successful applications of conservation biological control is the establishment of permanent strips of natural vegetation within cereal fields, so-called “beetle banks,” to provide a long-term home for natural enemies (Box 5.2).

Aside from establishing a natural area within the crop, as with beetle banks, several totally different types of crop plants can be planted within the same field. This practice of polyculture, also called intercropping, is used in over 2.3 million ha in northern China to reduce damage in cotton from cotton aphids. Cotton and wheat are

Box 5.2 | Beetle banks

This method has been developed to create favorable habitats for predatory invertebrates within fields by providing “islands” of diversity. Hedgerows, which are rows of shrubs, formerly surrounded agricultural fields in the United Kingdom but these are now less common. Therefore, under current agricultural practices, edges of fields often provide little overwintering habitat for predatory invertebrates. With very large agricultural fields, it can take a long time before the predators that survived the winter at the field edge or in some permanent vegetation in the area invade the crop the following spring and they might not travel to the center of large fields, let alone feed there for prolonged periods.



Dimensions of grass ridges called “beetle banks” that create a permanent habitat for natural enemies of crop pests in cereal fields. (Courtesy of the Game Conservancy Trust, UK.)

So-called “beetle banks” have been used for a diversity of field crops and the goal is to control smaller, pestiferous insects by enhancing predator populations. A beetle bank is constructed by creating a ridge or bank of earth about 0.5 m high and 1.5–2 m wide, extending for most of the length of a field, using two-directional plowing. With larger fields, it may be necessary to construct more than one of these wide ridges but this would depend on the dispersal ability of the major predators present. In areas where beetle banks are used in the United Kingdom, a 20 hectare field will need one beetle bank. With this density of beetle banks, radiation of ground beetles from beetle banks in spring resulted in a uniform distribution within cereal crops after the bank had been established for 3 years (Thomas *et al.*, 1991). After establishing a ridge, it is planted in the autumn or the following spring with tussock- or mat-forming perennial grasses, such as cock’s-foot grass (*Dactylis glomerata*). The dense structure of this type of plant provides habitat for predatory invertebrates and, once established, excludes most weeds. It takes a while before a community of predators becomes established in a beetle bank. After 2–3 years, a beetle bank will have become a suitable home for overwintering insects and spiders and, as a result, by then the crop surrounding the beetle bank will host fewer pests. For studies conducted in south-central England, densities of predators increased to more than 1,600 per m² after 2 years for some banks (Thomas *et al.*, 1992); the most common predators were ground beetles and spiders. These man-made habitats appear to strengthen natural controls in the fragmented and unstable environments created by intensive farming.



Cock's-foot grass, *Dactylis glomerata*, a tussock- and mat-forming perennial grass recommended for use in beetle banks because it provides shelter for natural enemies. (Courtesy of the Game Conservancy Trust, UK.)

This is one of the few instances of widespread grower adoption of habitat manipulation tactics. Hundreds of beetle banks have been created in several northern European countries and the term “beetle bank” has been included in the *Oxford English Dictionary* (Landis *et al.*, 2000). Beetle banks have been used in numerous types of field crops having lower plant canopies, but seem especially appropriate for controlling aphids in cereal crops. The cost of a beetle bank has been estimated at approximately UK £85 for a 20 hectare winter wheat field during the first year when the ridge is created and sown with grass seed and yield is lost for the area that is becoming a beetle bank. In subsequent years, the cost of a beetle bank is UK £30 per year, due to the yield lost from that area of the field. On the other hand, if no pesticide sprays are necessary, UK £300 would be saved per year in labor and the cost of pesticides, and if yield loss in fields due to aphids is prevented, a grower would be saving UK £660 per year. Long-term studies have shown that after establishment, costs of maintaining the beetle bank are low because only periodic mowing is required every few years (Landis *et al.*, 2000).

One of the driving forces behind the widespread construction of beetle banks has been to control pestiferous insects while reducing pesticide use. It is thought that beetle banks will also help to rebuild the declining populations of game and song birds. A side-effect of beetle banks has been that they provide nesting habitat for birds and sources of food for chicks. Work on improving beetle banks continues, especially with additions of plants that act as nectar sources alongside beetle banks (Landis *et al.*, 2000).

interplanted and natural enemies are thereby maintained in wheat fields. Wheat grows first and natural enemies feed on prey in wheat but as cotton grows, the natural enemies then move into cotton when prey are present. Without wheat as an alternate habitat, once cotton

begins to grow, predators will eventually arrive but usually they arrive too late to control aphid populations adequately.

Traditional crops for resource-poor Mexican and Central American farmers are frequently corn, beans, and squash, interplanted in the same small fields for subsistence. With this diverse vegetation, predatory ants feeding on a broad diversity of prey have been reported maintaining control of a variety of pest species (Perfecto & Castiñeiras, 1998). Studies in Nicaragua and Mexico documented that several species of ants were responsible for controlling fall armyworm, *Spodoptera frugiperda*, and corn leafhopper, *Dalbulus maidis*, on corn foliage, and rootworm eggs in the soil. Two studies excluding ants from plots, found much higher crop damage in plots without ants.

In greenhouses where pests are managed using natural enemies, pollen and nectar-bearing flowering plants maintained among the vegetables or ornamentals provide alternate food for the predators and parasitoids that are released (B. Bell, pers. commun.). Especially if an inundative strategy is used with “blind releases” of natural enemies on a regular schedule when prey populations are very low, providing alternate food helps to keep natural enemies that have been released alive for longer.

Cover crops

A dense plant canopy can also improve natural enemy populations by providing a sheltered microhabitat within the crop. Cover crops in citrus orchards in Queensland, Australia, are important for control of phytophagous mites. Between 80% and 95% of growers in the major citrus-growing districts encourage the flowering of Rhodes grass (*Chloris gayana*) during the fruit-bearing season because the grass pollen produced is used as alternate food by predaceous mites. To do this, alternate inter-rows between citrus trees are mowed every 3 weeks to allow time for production of pollen from grass growing between rows while still maintaining a neat orchard. In addition, 30–50% of growers plant *Eucalyptus* trees with hairy leaves in wind breaks so that pollen is caught on leaves and predators can build up long-term populations in these refuges (Landis *et al.*, 2000). This general type of approach is also widely used in China, where cover crops are present in an estimated 135,000 ha of citrus orchards to provide pollen for natural enemies of the citrus red mite, *Panonychus citri*.

As a caveat, cover crops are not the answer for all systems because in some cases, these plants can compete with the crop plants and decrease yield (van Driesche & Bellows, 1997). Cover crops can also provide resources for pests. In peach orchards, ground covers are often eliminated because they provide resources used by true bugs that feed on the peach flowers and fruit, resulting in scarring on peaches known as “cat-facing,” because the surface of the fruit at harvest resembles the face of a cat.

Crop residue management

Many parasitoids and predators inhabit crop residues after harvest and burning or removing these residues can decimate natural enemy

populations. Residues can be left in the fields, at least in part, to help conserve natural enemy populations. Several parasitoids attacking the sugarcane leafhopper, *Pyrilla perpusilla*, in India can effectively control pests if crop residues are not burned but are spread back onto fields.

Crop management

In California, both alfalfa and cotton are commonly grown and the pestiferous lygus bugs, *Lygus hesperus*, feed on both, although they prefer alfalfa. Alfalfa is harvested several times each year and, when an entire field of alfalfa (= lucerne) is mowed during hot weather, the lygus bugs leave within 24 hours. Often they leave alfalfa and move to cotton where they can cause substantial damage leading to pesticide applications. This problem is clearly due to harvesting practices so new harvesting practices were devised (van den Bosch & Stern, 1969). If alfalfa is cut in alternating strips, then lygus will migrate not to cotton but to the non-cut alfalfa strips that are nearby. Thus, chemical pesticides are not sprayed on cotton and this preserves the resident natural enemies. This practice also preserves the resident natural enemies in alfalfa because these move to the non-harvested strips along with the lygus bugs. Unfortunately, this strategy was never widely adopted by growers because it was more expensive than standard practices. A strategy to interplant alfalfa with cotton was also proposed but this posed difficulties because these two crops have different water requirements and modifications of the water system and extra labor to cut alfalfa did not compensate for the reduced pesticide use. However, due to pesticide resistance and the fact that more insecticides are applied to cotton than any other crop, it has been suggested that these practices might now be practical (Bull & Menn, 1990).

During a monumental study in the hot Central Valley of California, Schlinger and Dietrick investigated whether there really were more natural enemies when alfalfa was strip-harvested. They had chosen a crop with an incredible biodiversity, there being more than 1,000 different insect species in an average unsprayed field of alfalfa in California. They sampled 4.2 m² of alfalfa every 2 weeks and showed that all natural enemies except green lacewings were more abundant in strip-harvested fields (Table 5.2). In summary, strip-harvested alfalfa had four times as many natural enemies as regularly harvested fields (Schlinger & Dietrick, 1960; Dietrick *et al.*, 1960). In addition, insecticides were not needed on strip-harvested alfalfa but had to be applied twice to the regularly harvested field.

Plant characteristics

If monocultures are grown, care can be taken to use cultivars of plants that enhance natural enemies. Extensive studies in greenhouses showed that the whitefly parasitoid *Encarsia formosa* is very effective on numerous vegetable crops, but it consistently did poorly against whiteflies on cucumbers. The main factors for poor activity

Table 5.2 | Natural enemies associated with strip- versus regularly-harvested alfalfa throughout the field season, Kern County, California.

	Strip-harvested	Regularly harvested
Spiders	1,094,000	105,000
Parasitic wasps	287,000	70,000
Big-eyed bugs (predators)	401,000	199,000
Lady beetles	437,000	57,000
Green lacewing larvae	206,000	195,000

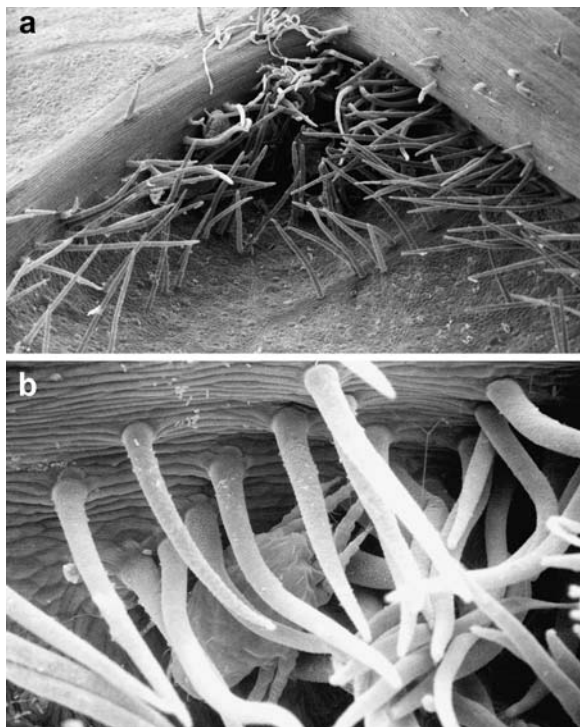
From (Schlinger & Dietrick, 1960)

were that cucumbers are a good host plant for greenhouse whitefly so the pest grows very fast. However, of equal importance, the cultivar of cucumber that was regularly planted had large leaf hairs that reduced the walking speed of the parasitoid. The hairs also caught the sticky honeydew from the whiteflies (the sugar-rich liquid excreta produced from whiteflies, aphids, scale insects, and mealybugs) and if the parasitoids contacted the honeydew, they would become stuck to it (van Lenteren & Martin, 1999). This situation was solved by plant breeding to develop a cultivar with half of the leaf hairs that were present on the commercial cultivar.

In contrast to the negative impact of leaf hairs described above, leaf hairs (trichomes) can enhance the abundance of beneficial phytoseiid and tydeid mites. Phytoseiid mites are predaceous and are used as biological control agents for control of plant-feeding mites. In perennial cropping systems, persistence of these predators is a key to successful biological control and abundance and persistence of phytoseiids is often greater on plant species and plant cultivars that have many leaf trichomes. These trichomes protect the beneficial mites from other predators and enhance the capture of pollen that often is eaten if pests are not present. Furthermore, when on plants with leaf trichomes, predatory mites are less prone to leave the plant. On some crops such as plums and pears that lack leaf trichomes, mite biological control has generally not been successful. In contrast, in apples, where most cultivars have abundant leaf trichomes, mite biological control can be very successful. Among grape cultivars, there is wide variability in leaf trichomes and data suggest that the success of mite biological control is at least in part associated with variation in trichome density.

Tydeid mites eat fungi and have been shown to suppress plant diseases such as powdery mildew caused by a fungal pathogen that predominantly lives on leaf surfaces. However, the effectiveness of tydeid mites hinges on the presence of tufts of leaf trichomes known as domatia that are used as refuges by numerous mites, including tydeids (Fig. 5.1). Domatia often occur at junctions in leaf veins. On some plants lacking these domatia, tydeiid mites are scarce and mildew is not suppressed.

Fig. 5.1 a. Domatium at the intersection of veins on a leaf of wild river grape, *Vitis riparia*. b. Tydeid mite, *Orthotydeus caudatus*, amongst leaf hairs within the domatium. The mite is approximately 0.2 mm long. (From Agarwal 2000, photo by Andrew Norton and Harvey Hoch.)



The waxiness of plant leaves can vary by plant cultivar and has been shown to affect natural enemies of herbivores. Predatory insects were released on cabbage cultivars with reduced amounts of wax covering leaves. Adult lady beetles, insidious flower bugs, and larval lacewings ate more diamondback moth (*Plutella xylostella*) larvae when hunting on low-wax cabbage leaves. These predators required much more time walking on waxy leaves because wax particles attached to their feet and they spent time either scrambling for attachment or grooming (Eigenbrode *et al.*, 1996) (Fig. 5.2). More recently, Patrick Duetting (2002) found that more pea aphids (*Acyrtosiphon pisum*) became infected with the fungal pathogen *Pandora neoaphidis* on low-wax pea leaves; companion studies suggested a mechanism by which fewer of the spores of this entomopathogenic fungus adhered to waxy leaves so there was less inoculum to infect aphids on waxy-leaved plants.

At present, the plant cultivars favoring natural enemies described above, grape cultivars with increased hairs on leaves and cabbage and pea cultivars with glossy leaves, are not being used by growers. This is recent research and the next steps must be taken with field trials to demonstrate that these cultivars will not be more susceptible to other pests and are equally productive under field conditions.

Soil

Soil can frequently function as a reservoir for natural enemies but we know much less about what goes on under ground than above

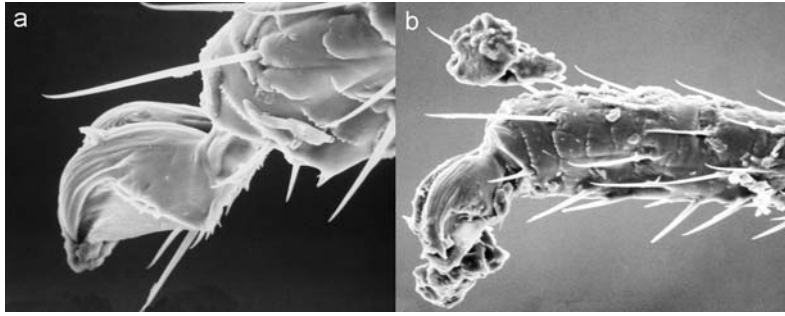


Fig. 5.2 Scanning electron micrograph of the tarsi of adult insidious flower bugs, *Orius insidiosus*, that had walked for one hour on leaves of cabbage cultivars with a. glossy leaves or b. normal wax leaves. (Eigenbrode *et al.*, 1996.)

ground. Suppressive soils that control plant pathogens and plant parasitic nematodes are well known (see Chapter 16) and soils can sometimes be manipulated to build suppressiveness in non-suppressive soils. Through planting the same crop on the same fields for numerous years, non-suppressive soils can sometimes become suppressive.

The soil acts as a reservoir for microbes, including microorganisms that are pathogens of invertebrates. Research has shown that tillage of the soil can alter the ability of this reservoir to infect pests. When fields are not tilled, spores of fungi infecting insects are at much greater titers at the soil surface where they will contact pests but, with tillage, the spores can become buried where few hosts are present. In a similar example, velvetbean caterpillars feeding on grass in pastures are more abundant when fields are tilled because the viruses infecting them are buried during tillage (see Fuxa, 1998). Soil can also serve as a reservoir for arthropod natural enemies. Numerous parasitoids utilize the soil for pupation and their populations are lower in tilled areas than untilled. For example, densities of parasitoids of rape pollen beetles were 50–100% lower when rape fields were plowed (Nilsson, 1985).

Physical environment

The physical environment strongly influences the activity of natural enemies. Application of water has been used to improve the microclimate within crops and enhance pathogens of insect pests. In greenhouses, activity of the fungus *Verticillium lecanii* can be enhanced by watering and providing night-time temperatures that yield the high humidities necessary for infection. Altering plant density also can be used to increase humidity in the microclimate occupied by pest and pathogen. Infections by the fungus *Nomuraea rileyi* in three species of caterpillars were greatest when soybeans were planted early, in narrow rows with high seeding rates so that the plant canopy closed early, thus increasing the relative humidity in the microhabitat occupied by both host and pathogen (Sprenkel *et al.*, 1979). The fungal pathogen *Zoophthora phytonomi* and its alfalfa weevil host (*Hypera postica*) have been manipulated to enhance disease epizootics. If alfalfa is cut early but left in rows, alfalfa weevils aggregate within the rows of harvested alfalfa. The microclimate within the windrows sitting in the sun is warm and humid and under these conditions this

fungus causes high levels of infection among the crowded beetles. Models demonstrated that early-season insecticide decision thresholds, early harvesting, and relying on this fungus to decimate weevil populations instead of applying insecticides could increase profits by as much as 20% (Brown, 1987). This program was validated during field trials and recommended for alfalfa weevil management in the state of Kentucky.

5.2.3 Enhancing habitat for natural enemies: using the area around the field

One of the best-known strategies for conserving and enhancing natural enemies is to provide “wild insectary” areas at the edges of fields of cultivated plants. These areas of “companion plants” can serve to provide food and shelter when there is no crop in the field or if the crop does not provide the resources needed by the natural enemy. These areas are much more effective if they are present over the long term so that natural enemy populations can build in them. One well-known example is the use of flowering plants along the edges of agricultural fields to provide nectar for parasitoids and pollen for predators. To this purpose, in Switzerland, “weed strips” of native flowering plants are frequently maintained in and around fields. Densities of numerous predators (ground beetles, predatory flies, damsel bugs, and spiders) increase when weed strips are present (Landis *et al.*, 2000).

Several species in the plant genus *Euphorbia* naturally grew as weeds around sugarcane fields in Hawaii. These plants provided nectar and mating sites for adults of a tachinid fly (*Lixophaga sphenophori*) that parasitized the sugarcane weevil, *Rhabdoscelis obscurus*. When herbicides were applied to ditch banks and field edges, these plants were all killed and there was a correspondingly great decline in the parasitic fly populations. Once it was recognized that these flowering weeds were important, growers altered herbicide applications to spare these weeds (Topham & Beardsley, 1975).

Larvae of hoverflies, or syrphids, can be important predators of aphids. Adults need nectar for energy and pollen for sexual maturation. A flower from dry areas in the North American southwest, *Phacelia tanacetifolia* (Fig. 5.3a), was planted alongside winter wheat fields in the United Kingdom and syrphid populations were monitored. More syrphid eggs were laid and fewer aphids were found in wheat field surrounded by flowers (Hickman & Wratten, 1996). Researchers have investigated other plants as pollen and/or nectar sources for other natural enemies. However, precautions have to be taken that the pollen and nectar that are provided do not lead to increases in pest populations. For example, when sweet alyssum, *Lobularia maritima* (Fig. 3b), was planted alongside lettuce to build populations of predators and parasitoids that would attack aphids, leafminers were also attracted by this nectar source (Chaney, 1998).

In California vineyards, the pestiferous grape leafhopper, *Erythro-neura elegantula*, can be successfully controlled by the tiny egg

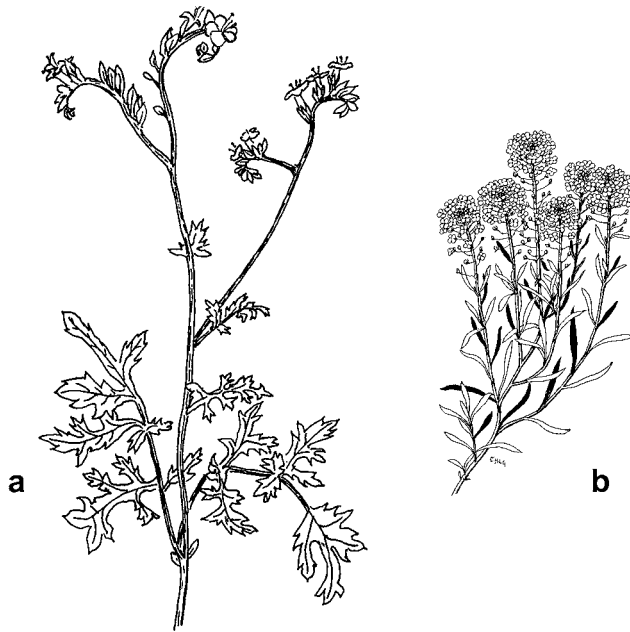


Fig. 5.3 Two species of flowering plants used to provide nectar and pollen alongside fields of crops. a. Lacy phacelia, *Phacelia tanacetifolia* (Leake *et al.*, 1993). b. Sweet alyssum, *Lobularia maritima*. (Courtesy of the Bailey Hortorium Herbarium, Cornell University.)

parasitoid *Anagrus epos*. However, this parasitoid cannot overwinter in grape leafhoppers because the grape leafhoppers spend the winter as adults. Finding high levels of parasitism near streams, researchers discovered that this egg parasitoid can overwinter in the eggs of blackberry leafhoppers, *Dikrella californica*, occurring in this habitat. Because streams did not run through all vineyards, a clever alternative was found. This parasitoid could also overwinter in eggs of the prune leafhopper (*Edwardsiana prunicola*) that feeds on leaves of French prune trees. Thus, when French prune trees occurred alongside vineyards, the parasitoids could overwinter in the immediate vicinity of vineyards and lower leafhopper populations resulted (Murphy *et al.*, 1998).

5.2.4 Providing food for natural enemies

Within crops or alongside crops, vegetation can be manipulated to foster natural enemy populations through planting specific plants to provide nectar and pollen or alternate hosts or prey for natural enemies. However, food can also be directly supplied to bolster natural enemy populations. Natural enemies require carbohydrates for energy and protein for growth and reproduction and these nutrients can be limiting in simplified monocultures. To bolster populations of lacewings eating aphids in cotton crops, Hagen *et al.* (1970) applied a mixture of protein hydrolysate, water and sugar to enhance lacewing reproduction. In addition, studies have suggested that artificial application of pollen can increase population densities of predatory mites and thereby increase the impact of these predators on pestiferous mite species (Van Driesche & Bellows, 1996).

5.2.5 Providing shelter for natural enemies

Protective habitats have been supplied in numerous systems to enhance natural enemy populations. These can serve as sheltered resting locations when the natural enemies are active but can also provide longer-term shelter during the winter. Some examples of such structures are polyethylene bags provided as nesting sites for predatory ants in cacao plantations in Malaysia, boxes for wasps and overwintering lacewings, empty cans in fruit trees for earwigs, straw bundles for spiders in early-planted rice and cotton or leaf litter around tree trunks as overwintering sites for predatory mites in apple orchards (Van Driesche & Bellows, 1996).

FURTHER READING

- Barbosa, P. (ed.). *Conservation Biological Control*. San Diego, CA: Academic Press, 1998.
- Bottrell, D. G. & Barbosa, P. Manipulating natural enemies by plant variety selection and modification: a realistic strategy? *Annual Review of Entomology*, **43** (1998), 347–367.
- Dennis, P. & Fry, G. L. A. Field margins: can they enhance natural enemy population densities and general arthropod diversity on farmland? *Agriculture, Ecosystems and Environment*, **40** (1992), 95–115.
- Landis, D. A., Wratten, S. D. & Gurr, G. M. Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology*, **45** (2000), 175–201.
- Pickett, C. H. & Bugg, R. L. (ed.). *Enhancing Biological Control: Habitat Management to Promote Natural Enemies of Agricultural Pests*. Berkeley, CA: University of California Press, 1998.

Part II

Biological control of invertebrate and vertebrate pests

Among the pests covered in this book, animals with and without backbones are often extremely obvious to humans. Vertebrates are animals with backbones while invertebrates are all multicellular animals without backbones, thus including a broad array of organisms from worms to dragonflies to lobsters. While both vertebrate and invertebrate animals can be pests that need to be controlled, they differ in the diversity of problems they cause as well as in the potential means for using biological control against them.

Invertebrates

The invertebrates covered in the following chapters will principally include insects and mites, both belonging to the jointed-legged animals, the arthropods. Plant parasitic nematodes are the focus of biological control efforts but will be covered in Chapter 16 and 17 because they are historically most commonly considered along with plant pathogens. Among the invertebrates, the arthropods targeted most tirelessly for biological control are pests in terrestrial systems, although some aquatic pests are targeted. It has been estimated that there are from less than 5 million to as many as 80 million species of insects alone. Such a huge number of species is accompanied by a great diversity in life-history strategies.

Controlling pestiferous insects and mites has most certainly always been a concern of humans. However, it is difficult to quantify the injury or damage incurred by insects around the world. In 1997, it was estimated that in the USA alone each year \$7.7 billion of crops are lost due to insects. There are certainly many other problems caused by insects and this figure does not include the costs of direct effects of

arthropod pests on human health and welfare or effects on the health of other animals. Insects also have a huge impact because they vector some diseases of humans, other animals, and plants. In addition, insect pests can have profound effects on the native flora and fauna, thereby altering natural ecosystems.

A great diversity of natural enemies has been used to control pestiferous insects and mites. Predators and parasitoids (insects parasitizing and killing other insects) are considered macro-natural enemies. Many of these macro-natural enemies are themselves insects and mites, so while some insects and mites are pests, others are natural enemies. Among the microorganisms, bacteria, viruses, fungi, and a diversity of single-celled organisms including microsporidia all can be important natural enemies, causing disease in insects and mites.

Vertebrates

Around the world, vertebrates have been introduced to many new areas where they have become pests. Some introductions have occurred naturally, such as range expansion of a species, or accidentally, while in other cases vertebrates were introduced intentionally for meat or fur, recreational hunting, and fishing. It has been estimated that in the USA, 50% of the animal species that were originally deliberately introduced as pets have become pests. Unfortunately, in a few early and misguided instances, vertebrates introduced for biological control have actually become pests (but see Chapter 18).

On the whole, vertebrate pests are more intelligent and adaptable than microbial, plant, and invertebrate pests and this adaptability makes them difficult to control. The most commonly used methods for control of pestiferous vertebrates are chemical control using poisons and cultural control by trapping, fencing, and shooting. These methods are expensive and provide only temporary solutions in localized areas.

Biological control programs have seldom targeted vertebrate pests compared with other types of pests and, among the vertebrates, mammals have been the focus of the majority of efforts to date (Hoddle, 1999). Lack of development of biological controls against vertebrates could be due, in part, to the fact that early attempts utilized the most obvious natural enemies, vertebrate predators. Most releases of vertebrate predators have had negative effects on the native non-target wildlife, especially on islands where there are few generalist predators. However, in one instance, an introduction of predators aided in control. As part of one success story, rabbit populations in Australia and New Zealand that are at lower densities due to biological control, are kept under further control by European foxes, ferrets, and cats (Newsome, 1990). Interestingly, high-density rabbit populations must be lowered, either by poisoning or by disease, before predators can be effective at maintaining lower population densities.

Few programs have investigated biological control of vertebrates using parasites, such as helminths, lice, ticks and fleas, because these natural enemies generally do not kill hosts. However, studies have shown that parasites of vertebrates can have a profound effect on host population densities (Dobson, 1988). In fact, vertebrates that have been introduced to a new ecosystem frequently arrive with few parasites and this probably aids in their success in colonizing the new area. Among the diversity of types of natural enemies, viruses are the major group used for biological control of vertebrates, but they have only been used against rabbits in Australia.

Chapter 6

Ecological basis for use of predators, parasitoids, and pathogens

Ecology may be the most intractable legitimate science ever developed.

(Slobodkin, 1988)

The ultimate goal of biological control is to manipulate systems to maintain pest populations at low densities and thus prevent problems due to pests. It follows that biological control in long-lived ecosystems can be thought of as a type of “applied population dynamics” (Murdoch & Briggs, 1996). In fact, studies of natural enemies and their hosts have been the basis for many ecological studies investigating population regulation. The field of ecology has gained from this association but it has been questioned whether ecological theory, in turn, has helped biological control. There is indeed a new movement to try to use insights gained from ecological theory to help increase the success of biological control.

Information from the majority of population dynamics studies of natural enemies and their interactions with their hosts can have relevance to classical biological control. Information from these studies may also be relevant to conservation biological control in providing the information about correct conditions for optimization of activity of natural enemies. Inoculative releases are also dependent upon interactions between host and natural enemy, although not on a permanent basis, while basic theory on long-term dynamics of natural enemies and hosts may have little application to inundative releases, where initial releases are expected to control the pest. Because the vast majority of biological control of animals is focused on arthropods, we will discuss the ecological basis for biological control using arthropods as examples.

6.1 | Types of invertebrate pests

Invertebrates are ubiquitous and critically important members of ecosystems but, unavoidably, numerous diverse invertebrate life histories lead to competition with humans. Invertebrates eat plants grown

by humans for food, supplies, or amenities and destroy many types of items built or stored by humans. They can carry plant pathogens from one plant to another. Invertebrates destroy trees and shrubs in natural areas such as wetlands or forests and can thus change nutrient cycling in these areas. Invertebrates infest bodies of domestic animals as well as humans, either externally or internally, or they can simply bother us. Invertebrates can transmit diseases from one vertebrate to another, with some pathogens spread only by these agents. Some invertebrates living in fresh water have been targeted by biological control programs but the majority of programs deal with terrestrial invertebrate pests, often plant-feeding pests.

When invertebrate pests are attacked by predators, they are referred to as prey and when attacked by parasitoids or infected by pathogens, they are referred to as hosts. For simplicity, throughout the following discussion, the terms host and prey are both used to describe ecological relations of natural enemies.

6.2 | Types of natural enemies

The natural enemies used to control invertebrates are taxonomically as well as functionally diverse. They include the functional groups of parasitoids, predators, and pathogens. Taxonomically, groups of natural enemies that are used for biological control range from fish to insects, mites, nematodes, and microorganisms, including bacteria, viruses, fungi, and single-celled organisms. Different groups of natural enemies are emphasized for different control strategies. Classical biological control and conservation have predominantly used insect parasitoids and predators and sometimes mites, while all types of natural enemies have been used inundatively. It would be far easier as well as more efficient always to use the same type of natural enemies, but not all groups of natural enemies have members that could provide effective control for each pest. Therefore, biological control practitioners must have training so they can work with different types of natural enemies.

There is one main goal in biological control relative to interactions between natural enemies and their invertebrate pest hosts. This is killing the individual pests as quickly as possible, while preventing further damage or injury to the greatest extent possible. Death of the pest can be rapid as with an attack by a predator or can be slower, when time is required as the natural enemy keeps the pest alive as a source of food. Of course, for classical biological control and conservation as well as for inoculative releases, it is important for natural enemies to reproduce before they die, usually using the bodies of pests as food. In contrast, for inundative releases, reproduction of natural enemies is not expected.

6.2.1 | Natural enemy attributes

Early models suggested a number of general attributes characterizing successful biological control agents: (1) host specificity, (2) synchrony

with the pest, (3) high rate of increase, (4) ability to survive periods with few to no prey, and (5) good searching ability. Such properties are more important for classical biological control or conservation and are more characteristic of parasitoids than predators or pathogens. Based on these general attributes, generalist predators would be less-well-suited for classical biological control, because they have lower rates of increase and are frequently not synchronized with the pest. However, we know that predators can be successful in classical biological control. In fact, even parasitoids that have been successfully used for biological control do not possess some of these attributes.

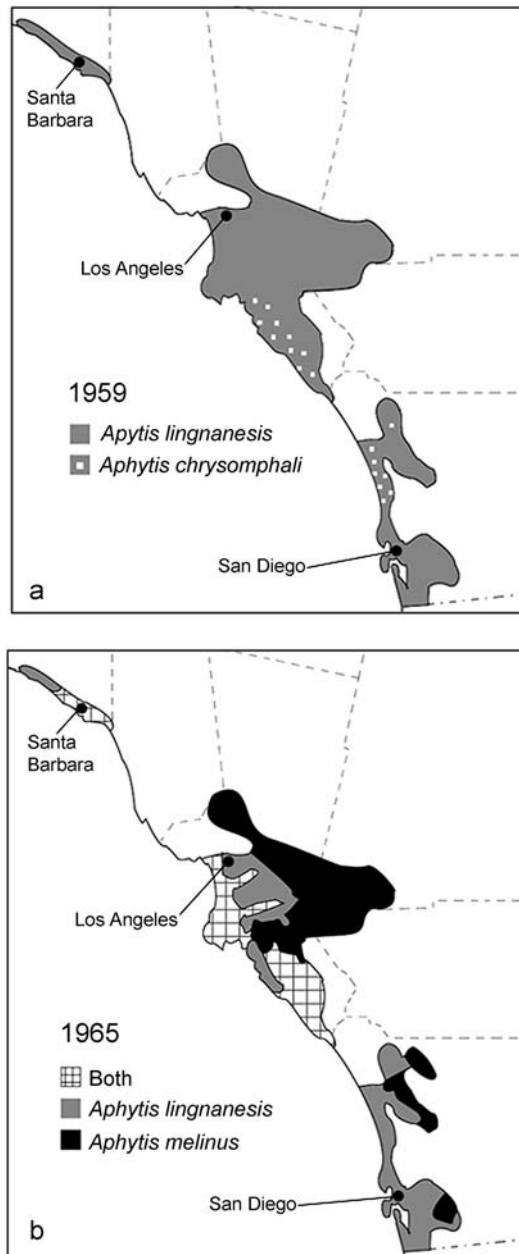
These attributes are quite general and research in particular systems has shown that seemingly minor differences in biologies can make big differences in efficacy for control. The parasitoids of California red scale, *Aonidiella aurantii*, introduced to southern California, provide an excellent demonstration of the variability in attributes of natural enemies associated with successful control. This scale insect occurs worldwide on citrus, feeding through the bark of trees, and can be a major pest. It was introduced to southern California some time between 1868 and 1875 and the first attempts to introduce natural enemies to control this scale were made as early as 1889. Several different species of parasitoids were involved. The first species, *Aphytis chrysomphali*, was probably originally introduced from the Mediterranean around 1900, and then spread across the distribution of citrus. It was not until *Aphytis lingnanensis* was introduced from southern China in 1948 that significant control was seen. By 1959, *A. lingnanensis* had spread throughout the citrus-growing area and the distribution of *A. chrysomphali* had become limited to specific areas (Fig. 6.1a). However, scale control was still inadequate in the hot, interior valleys of southern California. Therefore, the introduction program continued and *Aphytis melinus* was introduced from India and Pakistan, successfully providing control of red scale populations in interior valleys. *A. lingnanensis* could no longer be found in interior valleys; *A. melinus* had completely displaced *A. lingnanensis* in the interior regions where *A. lingnanensis* had not been effective (Fig. 6.1b). It became clear that *A. lingnanensis* was dominant in more humid climates with more even temperatures and *A. melinus* was dominant in drier climates with more extreme temperatures. Although under control, the scale remained present throughout its distribution at low and constant densities so that natural enemy populations could persist.

Ecologists have been very interested in this case of competitive displacement, asking what attributes were critical to the success of *A. melinus*. In the interior valleys, *A. melinus* displaced *A. lingnanensis* very quickly, within 1–3 years. Curiously, these two species are extremely similar morphologically and can only be distinguished in the pupal stage. Laboratory studies showed that *A. lingnanensis* was a better searcher and, when larvae of the two species occurred within an individual scale, *A. lingnanensis* outcompeted *A. melinus*, although parasitism by both species in individual scales was rare in the field. *A. lingnanensis* might suffer higher mortality in the more extreme climates of the interior valleys but this finding was not enough to

Fig. 6.1

Distribution and relative abundances of three species of parasitoids attacking California red scale in southern California.

a. *Aphytis chrysomphali* had previously occurred throughout this area but, by 1959, had been almost completely displaced by *Aphytis lingnanensis*. (Redrawn from DeBach & Sundby, 1963.) b. *Aphytis melinus* had spread and displaced *A. lingnanensis* in interior areas, while *A. lingnanensis* was predominant in more coastal areas and *A. chrysomphali* was only found in one location (not shown). (Redrawn from DeBach et al., 1971.)



satisfy biologists in explaining the observed patterns. One detailed difference was eventually noticed and used in a model to investigate its effect on competition between these two species. As with other parasitic wasps, *Aphytis* females can control the sex of offspring when eggs are laid (see Chapter 8). Generally, male eggs are laid in smaller scales and female eggs are laid in larger scales. *A. melinus* had an advantage because female eggs are laid in smaller scales than would be acceptable by *A. lingnanensis*. In the largest scales sometimes two

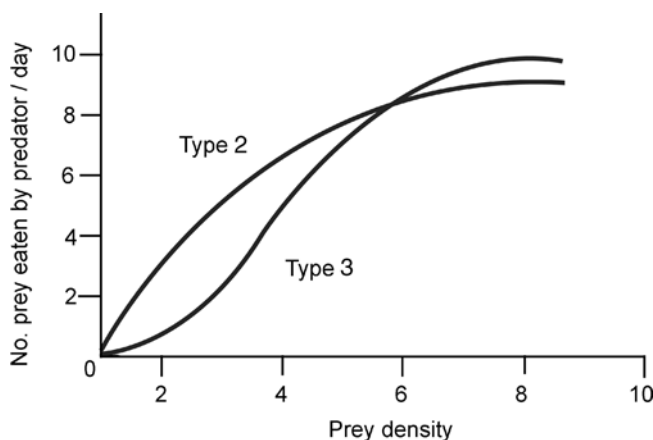
female eggs are laid by *A. melinus* when *A. lingnanensis* would lay only one. Therefore, *A. melinus* was able to produce more offspring using the same scale population. Adding these subtle differences in biology to a mathematical model of this system was enough to account for the rapid displacement of *A. lingnanensis* by *A. melinus*. These results also fit standard competition theory, which would predict that the winner of the competition would be the species that most reduces the equilibrium abundance of the common limiting resource, in this case the California red scale.

6.3 Interactions between natural enemies and hosts

Biological control does not occur when a few hosts are killed but rather when groups of hosts are killed and their populations remain low. Therefore, it is a phenomenon occurring at the population level. Studying populations that vary in space and time is typically more difficult than studying individual organisms. Progress has been made by studying individuals under controlled situations, followed by controlled studies (often experimental) of combinations of natural enemy and host individuals in the laboratory and in the field. Information on outcomes of studies has been used to derive mathematical models, created to help provide answers about the interactions that cannot be directly gleaned from data collected in the field. This type of approach is required because data from the field are typically influenced by many factors and their complex interactions, and one cannot readily see which are the key factors driving the observed situation. Experiments using mathematical models have been used extensively to investigate the emergent properties of groups of factors acting together. However, models are very sensitive to the assumptions used when building them, so starting from a good understanding of a system is critical.

Although there are numerous types of natural enemies, early work in developing ecological theory centered around interactions between predators and prey. An important interaction to be dissected was the response by predators to changes in prey density. Holling (1966) was instrumental in investigating the changes in predator behavior in response to changes in prey density that he called the functional response. The functional response is the behavioral response of predators to host density and should be differentiated from the numerical response, which involves increasing reproduction in response to prey density. Holling found that as prey density increased, the number of prey eaten increased quickly at first but then slowed, to eventually reach a plateau at satiation (Fig. 6.2, Type 2). Creating models for this response helped to identify the important components: (1) the rate of successful search (or rate of discovering prey), (2) the time available for searching, (3) the handling time (the time it takes the predator to eat that prey item and then be ready to search for another) and

Fig. 6.2 Two types of functional responses by predators to changes in prey abundance, with satiation at high prey densities.



(4) predator hunger. This functional response was subsequently found to be characteristic of many invertebrate predators and parasitoids. Response by vertebrate predators was characterized better by a sigmoid response (Fig. 6.2, Type 3). With frequent contact with prey, as would occur at higher prey densities, vertebrates could learn how to find, catch, and handle prey and thus respond more quickly, so the slope of the response was steeper although still reaching a plateau. Further studies showed that some invertebrates could also display sigmoid responses, especially those displaying more active searching in areas where more prey occurred.

Changing behavior when prey are more or less dense is only one component of a predator's response. A numerical response refers to the changes in numbers of predators when prey density changes. One can imagine an immediate increase in numbers of natural enemies as they gather at an aggregation of prey once it was discovered. For invertebrates, we also commonly see a more delayed response with increases in offspring following an abundance of prey or hosts as a result of increased reproduction. These concepts of functional and numerical responses are central to development of models describing interactions between predators and prey.

6.4 | Population regulation

When natural enemies control populations of prey or hosts this has been called population regulation. Populations are generally thought to be controlled by some combination of exogenous factors, factors external to the population such as the effects of natural enemies or climate, and endogenous factors, such as genetic changes in a population or intraspecific (within that species) competition (Table 6.1). Population regulation has been the subject of many studies and much discussion focused on understanding why natural systems maintain the structures we see. For our purposes, it is important to understand how pest populations are controlled by natural enemies to try

Table 6.1 Exogenous and endogenous factors interacting to regulate populations

<i>Exogenous</i>
Natural enemies (predators, parasites, pathogens)
Food supply
Weather
Shelter
<i>Endogenous</i>
Sex and age
Physiology
Behavior
Genetics

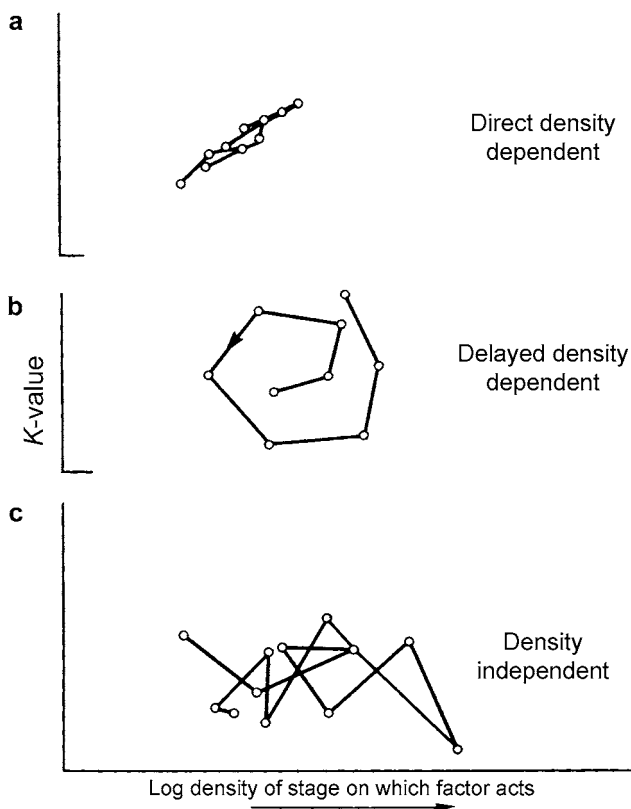
to improve biological control success rates. A key question concerns what governs the interactions between natural enemies and hosts to allow their coexistence. Why aren't natural enemies always able to kill all their prey? Several issues, including effect of the environment on natural enemies and pests, behavior of natural enemies and pests, responses of natural enemies to pest density, and actions of natural enemies and hosts on a spatial scale, are central to developing theories regarding how natural enemies coexist with their prey or hosts.

6.4.1 Density dependence

Central to the issue of regulation by natural enemies is the concept of density-dependent mortality, that mortality inflicted on members of a population which increases in relation to the density of the host or prey population (Fig. 6.3a). While this type of mortality would increase as the population increases it also decreases as the population decreases as a negative feedback. The decrease in mortality of the host at low densities is a critical attribute because in this way the natural enemy does not become extinct (but see below). This concept was central to models created by Nicholson and Bailey (1935), who believed that density-dependent factors regulated populations. Researchers studying natural enemies to try to fit them to models of density dependence soon found that data points often did not fall directly where expected. Instead of being density dependent, relationships are often 'density vague,' demonstrating that in reality, in all biological systems, responses are often variable but demonstrate general trends. Nevertheless, for many years scientists held that density-dependent responses by natural enemies to hosts or prey were required for successful biological control.

We can look at density dependence more closely, and classify it into different types of relations. In some systems, there is a time lag after an increase in host density and before mortality increases; this is called delayed density-dependent mortality (Fig. 6.3b). This can be characteristic of insect populations where a numerical response to

Fig. 6.3 Different relations between host or prey density and the mortality limiting the population. K values are used to quantify mortality in the host population. (After Southwood, 1978.)

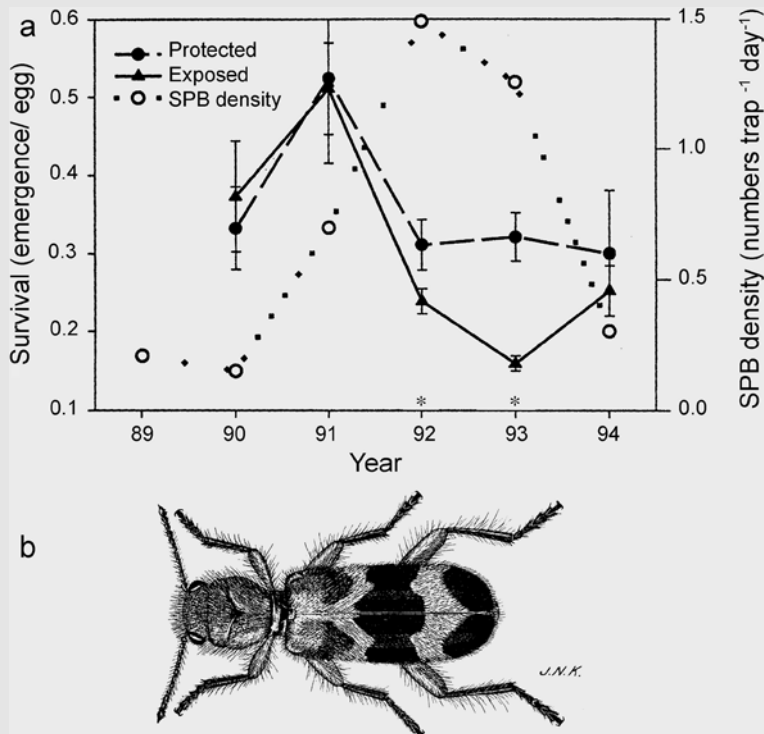


increasing host density requires time for a new generation of natural enemies to be produced. For southern pine beetles in loblolly pine stands in Texas, predator-caused mortality demonstrated a delayed response to southern pine beetle populations. Predator-caused mortality was negligible while bark beetle populations increased, then predators increased during the year that the pest population peaked and increased further the next year while the pest populations crashed (Box 6.1).

Box 6.1 | Bark beetle outbreak cycles explained

Populations of many animals are known to increase to outbreak densities that subsequently decrease and then build again, and this boom/bust cycle is thus repeated. Such population behavior is frequently called cyclic or oscillatory. In some cases, there is a periodicity to the cycles of increase and subsequent decrease but in many, increases seem to occur irregularly. The southern pine beetle is infamous for its periodic outbreaks that result in widespread economic damage as beetle-riddled pine trees turn brown and die. These tiny bark beetles (Scolytidae) use pheromones to mass attack trees where they lay eggs in galleries under the bark, often inoculating trees with tree-pathogenic fungi they carry, and their larvae subsequently damage the tree as they feed. It has been suggested that outbreaks in the southern USA are driven by climatic fluctuations. However, a 30-year study in

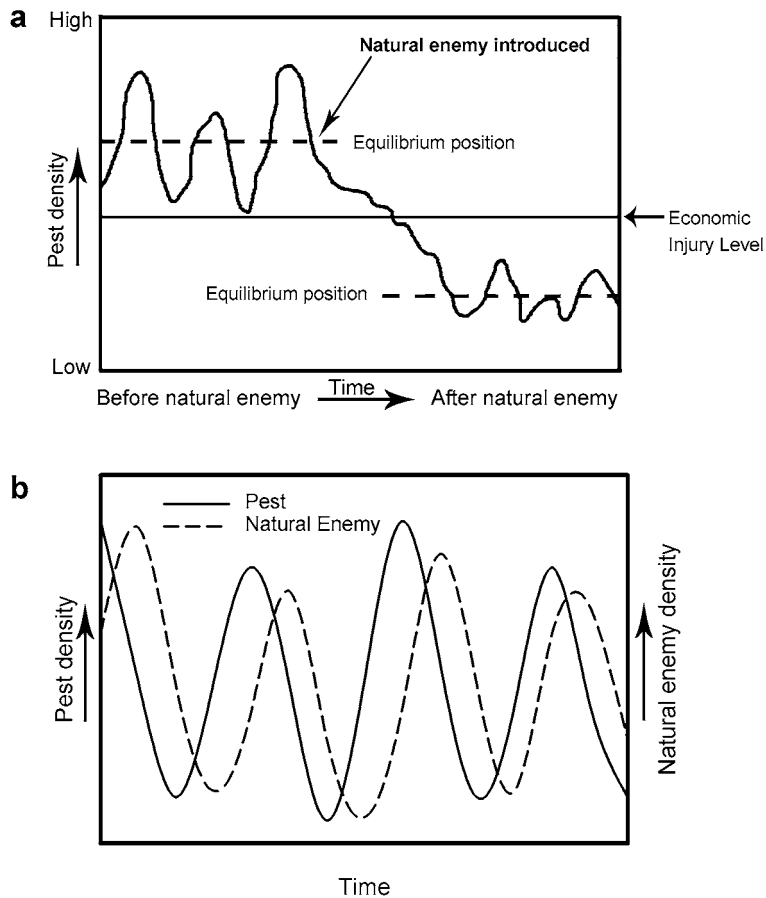
eastern Texas demonstrated that southern pine beetle population dynamics were associated with density-dependent factors, and especially factors acting with a delayed effect (Turchin *et al.*, 1999). To study these interactions, over a 5-year period, arthropod natural enemies were excluded from caged beetle-attacked trees and southern pine beetle numbers in these trees were then compared with numbers in uncaged control trees.



a. Survival of southern pine beetles (SPB), *Dendroctonus frontalis*, as measured by the proportion of eggs becoming adult, either from caged trees protected from predation or trees exposed to predation. Asterisks show where significant differences were found between densities in protected (caged) and exposed (uncaged) trees. The dotted line indicates the 6-year course of the bark beetle outbreak. (Turchin *et al.*, 1999.) b. The principle predator of southern pine beetle excluded from protected trees, was the checkered beetle *Thanasimus dubius*. (Illustration by Knull, 1951.)

Results showed that there were no differences in beetle populations between excluded and non-excluded trees when they were first attacked and beetle populations were building. The outbreak beetle populations subsequently decreased throughout the forest but not in the experimental trees, where predators had been excluded. Certainly, an entire guild of parasitoids and predators acted together to decrease the beetle outbreak throughout the forest, but these species had no access to the caged trees. One of the natural enemies found to be very abundant in uncaged trees during this study was a small red and black checkered beetle, whose larvae and adults both search under the bark in bark beetle galleries and specialize as predators of southern pine beetles.

Fig. 6.4 a. Hypothetical results of a classical biological control introduction in which the average abundance of a pest is reduced after introduction of a natural enemy, demonstrating stable equilibria both before and after the natural enemy is introduced. (From Flint & Dreistadt, 1998.) b. Hypothetical density-dependent relations in a predator–prey (or natural enemy–pest) system with discrete generations.



Of course not all mortality is associated with natural enemies or with host density. Density-independent mortality occurs without any relation to density (Fig. 6.3c). The classic examples of this would be when a weather event negatively affects a population, such as an early freeze causing extensive mortality among non-cold-hardy species, regardless of their density. Among early theorists, some felt that density-independent processes were extremely important and, for a time, the relative importance of density independence versus density dependence in determining host densities was a matter of great debate.

6.4.2 System stability

For biological control to be successful, it has long been thought that the natural enemy/host relationship must be stable. This meant that populations of the host would constantly be present and would fluctuate in density around some equilibrium density. After introduction of a natural enemy, that equilibrium density would decline to a new stable level at which natural enemy populations would track host populations (Fig. 6.4). In contrast, in an unstable system, fluctuations

could occur with resulting extinctions. Early models by Nicholson and Bailey used discrete generations with one generation of host and parasitoid per year but the results from this model were unstable, and fluctuated wildly through time before host and natural enemy became extinct. This model was based on encounters between host and parasitoid that occurred randomly. Once natural enemies in the model could search specifically for the host so that parasitoids could respond to high densities of the host, the results of the model became stable. However, this theoretical model was still very simple and homogeneous. When it was changed by introducing parasitoid movement, the model again lost stability. Researchers began to question whether this idea of a stable equilibrium was real. If interactions are in fact unstable, how is coexistence of natural enemy and host then achieved?

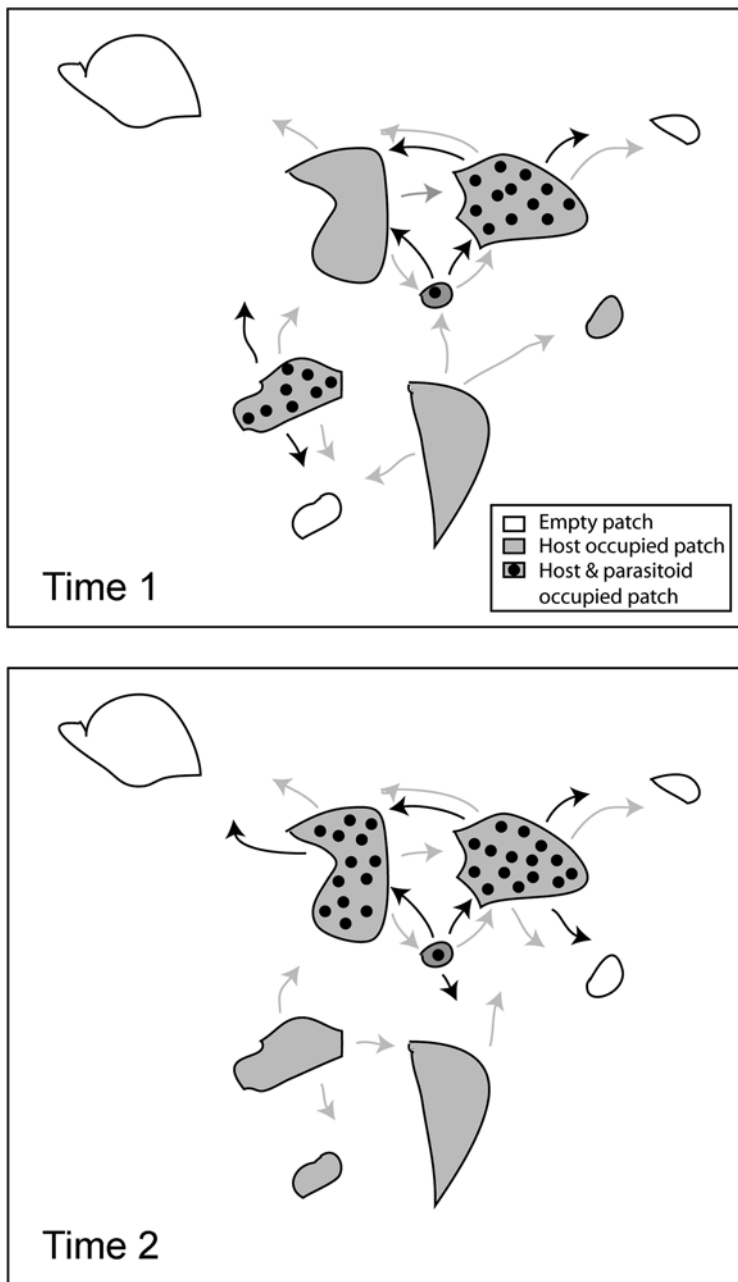
6.4.3 Host metapopulations

Zooming outward from those natural enemies attacking one population of a pest, we can gain insights looking at a larger scale, the metapopulation level. A metapopulation is a set of local populations connected to each other through dispersal. In reality, many species, unless at outbreak densities, have aggregated distributions. While host densities might decrease at one location, it is highly likely that they would not decrease throughout all patches of a metapopulation. A pest population could then all die in localized areas where there would then be no prey or hosts for the natural enemies. However, we know that under such circumstances, many if not most natural enemies would disperse. If dispersal occurs among local pest and natural enemy populations, then localized extinction of a pest would not especially result in extinction of that natural enemy in the larger area (Fig. 6.5). Therefore, the issue of stability of the system differs between local and metapopulation scales. A host/natural enemy combination could be unstable in a local population but on a regional scale could persist stably.

Elegant studies by Huffaker (Huffaker, 1958; Huffaker *et al.* 1963) were among the first to investigate metapopulation theory in the laboratory. Huffaker conducted studies using a mite that feeds on oranges and its associated mite predator by varying the environmental complexity of the system. He found that in simple systems with few oranges, the predators always found all of the prey and annihilated them, thereby causing extinction of both predator and prey (Fig. 6.6a). He added more trays of oranges with petroleum jelly barriers, creating metapopulations in this universe, but allowing some movement among oranges. In this way, prey and predator populations were maintained for 70 weeks (Fig. 6.6b), after which time the study was terminated. This study demonstrated that having a heterogeneous environment in which prey occurred as metapopulations could lead to system stability.

In systems where pests are phytophagous, the host plant, pest, and natural enemies often all occur in aggregated distributions.

Fig. 6.5 Hypothetical metapopulation dynamics showing patches that hosts could occupy and spatial distributions of host and parasitoid populations as they change through time, colonizing patches and disappearing from patches. (Illustration courtesy of Saskya van Nouhuys.)



In the field, metapopulations of herbivores have been shown to be interconnected by dispersal. In work with parasitoids, natural enemies often only occur in a subset of host populations. Natural enemies must arrive at sites after the host, therefore they are more constrained in their ability to colonize a new area successfully when dispersing, i.e., when dispersing, the herbivore only needs to find a patch of host plant while the parasitoid must find a patch of host plant where the herbivore already occurs. Certainly, this concept of

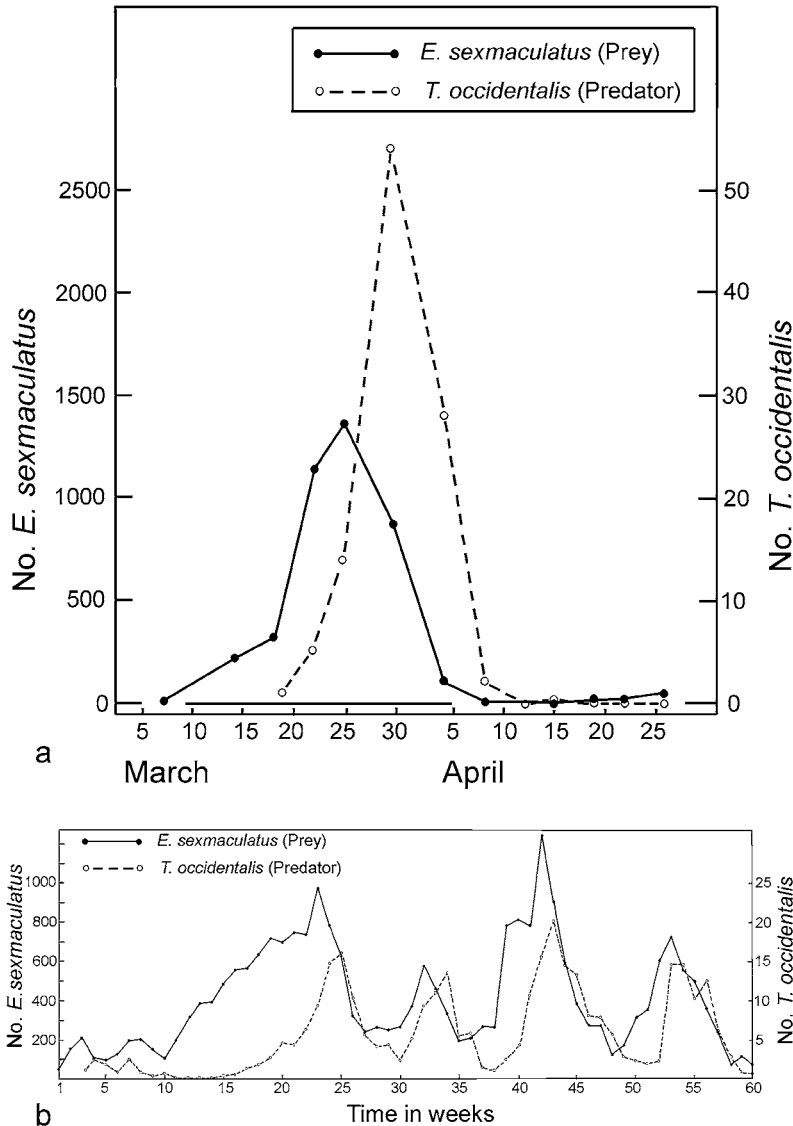


Fig. 6.6 a. Densities per unit area of orange for the prey mite *Eotetranychus sexmaculatus* and the predatory mite *Typhlodromus occidentalis* in a universe of 40 oranges, only 20 of which provided food for the prey. (After Huffaker, 1958.) b. Predator-prey interactions between *E. sexmaculatus* and *T. occidentalis* in a complex 252-orange system in which one-twentieth of each orange was exposed for possible feeding by the prey. (After Huffaker et al., 1963.)

metapopulations that account for localized extinctions is important to our understanding of the mechanisms by which natural enemies respond to and control pests in the field.

6.4.4 Refuges for hosts

As stated above, to achieve stability between natural enemy and host populations, both players must be present in an area. This can be accomplished by recolonization of spatially isolated patches after host extinction (as explained above) or, alternatively, by persistence of the host population in some way within the same area. If hosts have some sort of refuge where they cannot be killed, they can persist in that area. In a simple case, this could be a space in which the natural enemy could not reach the host. Alternatively, if the natural enemy is

omnivorous and switches to other food when the host is scarce, low-density host populations would be relieved from predator pressure because predators would feed on other prey. As with metapopulations, the relative importance of use of refuges by hosts in order to remain in an area is under study.

6.5 | Is stability necessary for coexistence of natural enemies and hosts?

A major question resulting about interactions between natural enemies and hosts has become: “To what extent does stability occur in these relationships?” By stability, we mean a relationship where numbers of the natural enemy and host fluctuate around some equilibrium density, with neither going extinct. The goal of biological control, of course, is for the equilibrium density of the pest to drop below the economic injury level (see Chapter 2). Early researchers considered that stability was necessary for classical biological control to be successful. Information from classical biological control programs only seemed to agree with the stability model.

However, while the early modelers considered stability of prime importance, in more recent years, Murdoch and Briggs (1996) have questioned whether a non-equilibrium model of interactions might be more realistic. In this scenario, density-dependent mortality does not have to occur and local populations of hosts can be unavailable to natural enemies (for example if using a refuge) or become extinct. In a non-equilibrium model, a stable equilibrium would not be necessary for biological control; pest populations in different local areas would fluctuate independently, sometimes perhaps wildly, but movements between these so-called “patches” would lead to stability on a metapopulation level. Data from several successful classical biological control programs were evaluated to test this idea (Table 6.2). Comparing four successful cases of classical biological control with the predictions from stability versus non-equilibrium theories demonstrated that the long-accepted dogma about a requirement of density dependence for successful biological control was in need of re-evaluation. Clearly, density dependence was not always associated with successful biological control.

To study how natural enemies regulate hosts, Murdoch and colleagues investigated interactions between the California red scale and the parasitoid *A. melinus* that keeps scale populations at low densities. They wanted to understand what was happening in this host/parasitoid relationship that seemed to defy the long-accepted dogma regarding the requirement of density dependence for successful biological control. Studies showed that these parasitoids were not density dependent in time; parasitism did not increase when scale populations were more abundant over time (Fig. 6.7). They were also not density dependent in space, i.e., higher parasitism was not seen on individual trees or branches having higher populations of scale (Fig. 6.8). Dissecting

Table 6.2 Comparison of two alternative models of successful biological control with examples from four case studies of parasitoids

	Stable equilibrium at low pest density	Density-dependent relation with host	Host-specific natural enemy	Natural enemy synchronized with host
Stability theory	Yes	Yes	Yes	Yes
Non-equilibrium theory	No	Not necessary	Not necessary	No
<i>Successful examples of classical biological control</i>				
Winter moth, <i>Operophtera brumata</i> , in Nova Scotia	No	?	No	?
Olive scale, <i>Parlatoria oleae</i> in California	No	No	Yes	No
Larch sawfly, <i>Pristiphora erichsonii</i> , in Manitoba	No	Yes	Yes	Yes
Red scale, <i>Aonidiella aurantii</i> , in California	Yes	No	Yes	Yes

Data from Murdoch *et al.* (1985); table after Krebs (2001).

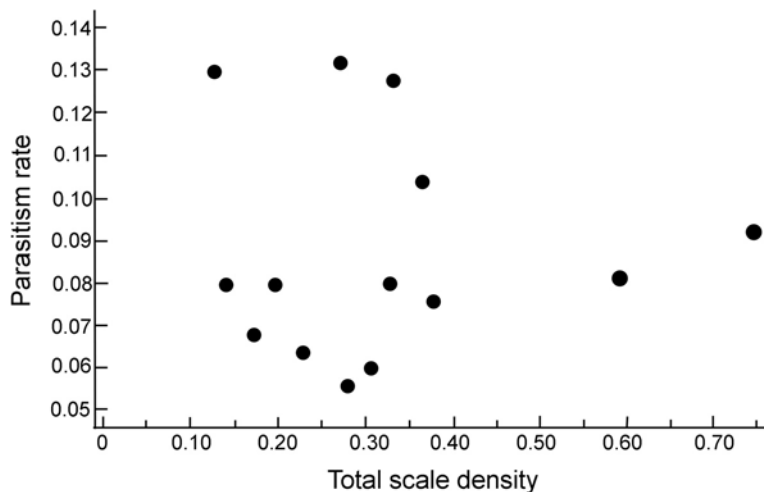


Fig. 6.7 Evaluating temporal density dependence by the parasitic wasp *Aphytis melinus* attacking California red scale on lemon trees over three years. The data do not fit a line that would demonstrate a density-dependent response of this natural enemy to the pest. (After Reeve & Murdoch, 1986.)

this system still more finely, Murdoch and colleagues found that, within each tree, there was a refuge. In the interior of each grapefruit tree, the scale density could be as much as 100 times greater than the scale density on the exterior branches of the trees (Fig. 6.9). It had been hypothesized that the Argentine ants, *Linepithema humile*, continually present on branches were disturbing parasitoids in the interiors of trees. By excluding ants from trees, more parasitism occurred in the interior of trees. However, parasitism still was not as great as at the exterior of the tree. Additional explanations for why the tree interior hosts higher scale densities is that the bark color of the interior branches is not attractive to *A. melinus*. Also, scales in the

Fig. 6.8 Evaluating spatial density dependence by the parasitic wasp *Aphytis melinus* attacking California red scale on lemon trees at three spatial scales, with no indication of density dependence in space. (After Reeve & Murdoch, 1985.)

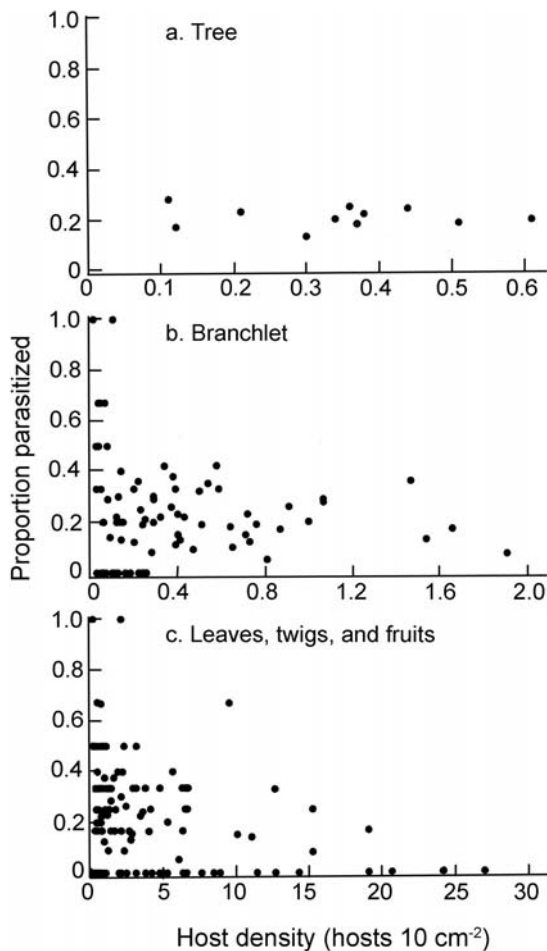
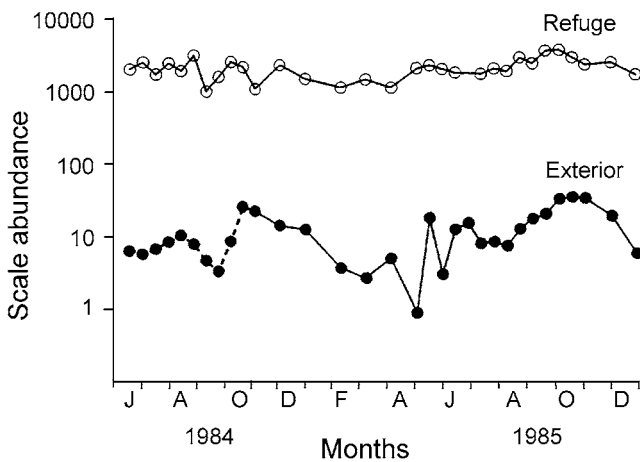


Fig. 6.9 Densities of California red scale per twig in the tree exterior and in refuge areas of grapefruit trees in southern California. The refuge area in the tree interior maintains red scale densities about 100 times the densities of those inhabiting the exterior of the tree. (After Murdoch *et al.*, 1995.)



tree interior are smaller and *A. melinus* prefers larger scales. Another source of protection for red scale is always present; those red scales reaching adulthood become safe from parasitism because *A. melinus* cannot attack adults. Thus, red scales have numerous ways to avoid parasite attack, both in space and in time, so that their populations remain higher in the tree interior serving as a source for recolonization of the tree exterior, where the parasitoids are very effective.

As a caveat, although Murdoch did not find density dependence of importance for regulation of California red scale, other population ecologists believe that density-dependent processes are very important in determining stability and persistence of populations. It is still thought by many that any process that acts in relation to pest density has a greater potential for stabilizing populations compared with a density-independent factor. However, the relative importance of different processes in regulating host populations is clearly still a matter of debate.

6.5.1 Allee effects

Lack of an equilibrium would mean that natural enemies and hosts would undergo local extinction, but how common is this? In the field, it is estimated that only 10% of introduced organisms become established and, in fact, many organisms purposefully introduced for biological control (which can be thought of as a special kind of invasive) do not become established. In some instances, as with accidental introductions of species that will potentially cause extensive damage, eradication is attempted by public agencies, to drive populations to extinction in the newly colonized area. The goal of such an eradication program is for the pest population to decrease to such a low density that the Allee effect is seen. The Allee effect is a phenomenon whereby fitness (ability to successfully reproduce) is correlated with population size. In particular, we are interested in the Allee effect seen when animal or plant species decrease to low densities and their rate of increase declines. This can also be thought of as inverse density dependence at low population densities that can drive populations to extinction. This effect can occur due to (1) failure to find mates in low-density populations, (2) failure to thrive at low densities if cooperation among individuals is needed, as among gregarious feeders, (3) inbreeding depression, or (4) efficient predators that are not satiated at low pest densities. Therefore, natural enemies do not have to find all hosts for the host population to become locally extinct due to the Allee effect. If hosts became extinct in an area, this does not especially mean that biological control will be ineffective. Metapopulation theory would suggest that an area could be recolonized, either by pest or natural enemy.

6.5.2 Responding to population increase

Parasitoids and predators are usually considered important for preventing pest outbreaks, but how do they respond quickly enough if

pests have such patchy distributions and systems are not stable, with local extinctions occurring? Two very different life history strategies of parasitoids and predators, both thought to allow natural enemies to respond to increasing host populations, have been called “search and destroy” and “lying in wait.” “Search and destroy” is employed by natural enemies that are highly host specific and are also good at searching for and finding their hosts. Spatial patchiness of the pest allows the pest to survive but these natural enemies eventually find and destroy individual pest populations, after which they disperse and search for another population of the host. This response is even better if the natural enemy develops faster than the host. Characteristics of natural enemies using this strategy, such as narrow host range, excellent ability to find hosts and high rate of numerical increase, have long been considered the goals for successful classical biological control agents such as *Aphytis* species controlling California red scale or *Vedalia* beetle controlling cottony cushion scale.

The “lying in wait” strategy is quite different, and is characteristic of populations of polyphagous natural enemies that are continuously present in local areas subject to pest infestation. When the pest is not present, these natural enemies survive for a time without food or by eating alternate food (sometimes including each other). These natural enemies thus persist in areas whether hosts are present or not and are present and ready to respond when the pest is once more present and/or beginning to increase. This type of response is characteristic of predaceous mites that keep phytophagous mites under control in orchards and vineyards; the effectiveness of these predators is evident once these predators are eliminated by pesticides and populations of phytophagous mite erupt.

6.6 Microbial natural enemies attacking invertebrates

Much of the theory regarding population regulation relative to biological control has principally been developed with parasitoids and predators in mind. However, we know that pathogens causing infectious diseases can be important natural enemies. Given free rein, many pathogens are known to be effective natural enemies, and can cause epizootics (unusually high levels of disease) in host populations (Fig. 6.10). Some attributes of the biology and ecology of microbes are different enough from parasitoids and predators that these should be mentioned. Microbes causing infectious diseases are highly variable in many attributes. Host/pathogen models, based on invertebrate pathogens, were developed with separate models for different sets of characteristics. Numerous microbes do not have mobile propagules and require healthy hosts to contact the pathogen. Other microbes have free-living stages with some means for transmission to a healthy

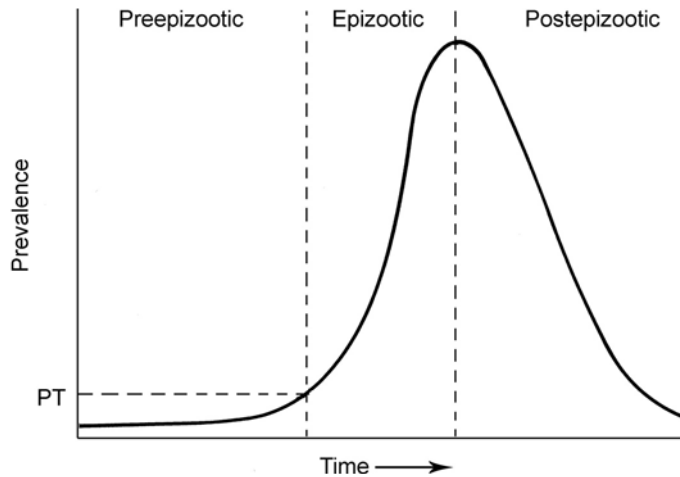


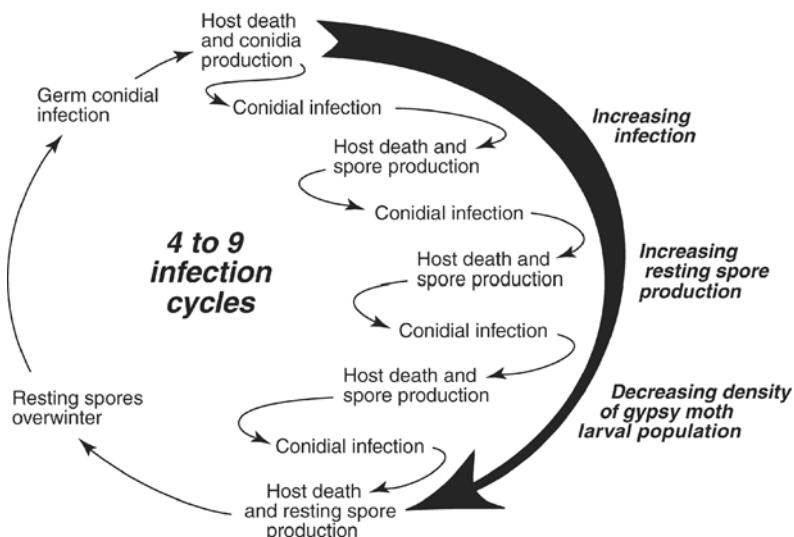
Fig. 6.10 The three general stages of an epizootic cycle demonstrating the increase and decrease in disease prevalence. The preepizootic phase occurs as long as prevalence is below a perception threshold (PT), followed by a dramatic increase in prevalence that is often short-lived relative to the other phases. The postepizootic phase occurs as disease prevalence decreases. (After Brown, 1987.)

host. Different pathogens may require vastly different numbers of propagules to achieve infection and, for some pathogens, hosts are not killed outright but the resulting chronic disease decreases reproduction.

As discussed above for predators and parasitoids, the occurrence of refuges for hosts can be important for pest regulation because some pests can remain in an area; the natural enemy then does not disappear at that location. Along these same lines, some microbes have long-lived stages that persist in the environment. Such stages are well known from various baculoviruses and fungi that infect insects; these often amass in the soil or the bottom of bodies of water. These locations can act as reservoirs where the pathogen persists in the environment. When pathogen propagules from such reservoirs infect hosts, this can be thought of as a first cycle of infection, often called primary infection. Primary infection can be followed by multiplicative cycles of infection, called secondary infection, when pathogen propagules produced from cadavers of recently killed hosts infect healthy hosts. Under optimal conditions, in this way pathogens can develop and kill hosts very quickly, and such cycles of infection can occur numerous times during one season (Fig. 6.11). Rapid increases in the numbers of hosts infected due to multiplicative cycles of infection occur (secondary cycling), resulting in levels of infection characteristic of an epizootic.

Simple models have suggested that pathogens of intermediate pathogenicity are more effective as biological control agents, while highly pathogenic microbes may contribute to wild episodes of increases (outbreaks) and decreases (due to epizootics) in host populations. With heterogeneity in virulence in the pathogen population, this tendency of the host and pathogen populations to cycle through outbreaks and epizootics could be dampened (Hochberg, 1989). Actual

Fig. 6.11 Multiplicative cycles of infection during one field season that create epizootics. Models have indicated that during epizootics in gypsy moth populations caused by the fungal pathogen *Entomophaga maimaiga*, four to nine infection cycles can occur during one gypsy moth generation. (Illustration by Frances Fawcett.)



data on dynamics of host populations have shown that a diversity of factors is always linked with cycles of pest outbreaks.

6.7 Food webs

Much of our discussion thus far has dealt with systems composed of one natural enemy and one host or, at most a few natural enemies and one host. However, in nature the interactions between these participants are only part of greater webs of interactions among many different organisms living within the same environment (Fig. 6.12). Systems can indeed become extremely complex and scientists have tried to classify them based on the major factors organizing the composition of the system. Systems where natural enemies seem to provide the major control of organisms feeding at lower trophic levels, such as lady beetles that control populations of aphids that feed on plants, are said to be “top-down.” Conversely, systems where the primary producers such as plants seem to organize the dynamics, such that the herbivore populations are not effective at reducing plant populations, are termed “bottom-up.” Unfortunately, although this categorization helps us think about factors driving interactions, as communities become more diverse (have more species) such simplified and directional control of dynamics is often not so evident.

For either type of system, top-down or bottom-up, there are often numerous species at each trophic (or feeding) level; these species coexist and often compete with each other. Organisms that utilize common resources in the same manner can be thought of as a guild. These species coexist and often compete with each other. Sometimes members of the same guild compete for the same resources. In an extreme case of competition, coexisting predators can eat each other

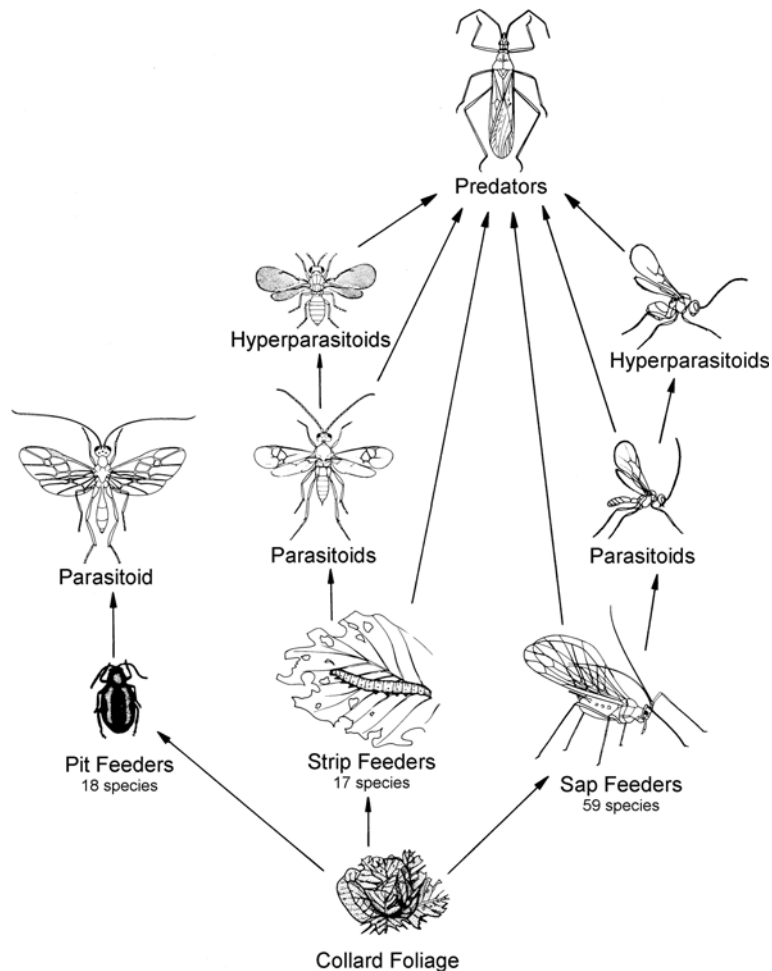


Fig. 6.12 The food web found on collards by Root (1973). This community contains three guilds of herbivores, the pit feeders, strip feeders and sap feeders, with parasitoids, hyperparasitoids and predators feeding on the herbivores and, to some extent, feeding on each other: (Price, 1984.)

(intraguild predation), as well as eating prey of different trophic levels, such as herbivores. Communities can be thought of as assemblies of many interacting guilds. This reduces the number of components in a community, thus facilitating their study.

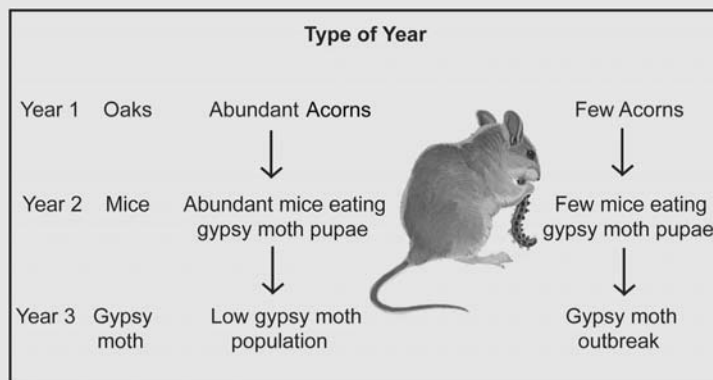
As one example of a guild, there are five major species of parasitoids that attack gypsy moth caterpillars in northeastern North America. This example demonstrates that guilds are not based on taxonomy but on ecological roles, because three of these parasitoids are tachinid flies and two are parasitic wasps. Within a guild, there is even division of labor through time, with succession in the species that are active. In the case of gypsy moth, one parasitic wasp attacks the eggs (*Ooencyrtus kuvanae*), another parasitic wasp attacks early instar larvae (*Cotesia melanoscelus*) and the three tachinid flies (*Compsilura concinnata*, *Blepharipa pratensis* and *Parasetigena silvestris*) kill late instar larvae. The three tachinids specialize further, with *C. concinnata* prevalent in low-density gypsy moth populations, *B. pratensis* most abundant at intermediate densities and *P. silvestris* most abundant during outbreaks.

Usually when classical biological control is initiated, the food web of concern is simple, with only a few natural enemies attacking an introduced herbivore. Effective top-down control in biological control is often due to a single parasitoid species, usually in a simplified system with an exotic herbivore feeding on an exotic plant in a cultivated habitat (Hawkins *et al.*, 1999). Instances of “natural control” (see Chapter 2) that have been documented often result from multiple links in more complex food webs. For example, populations of native insect herbivores on native plants in a natural habitat are often regulated by a guild of generalist predators (Hawkins *et al.*, 1999).

Frequently, species of several different trophic levels influence each other. This has been called tritrophic interactions when three trophic levels are involved. The host plant of a pest can affect a natural enemy, thus influencing the resulting population levels of the host. For example, if a specific plant provides resources that benefit the natural enemy such as protected refuges among leaf hairs (see 5.2.2), more natural enemies will be present, resulting in fewer hosts. Interactions between gypsy moth, mice and acorns demonstrate how several very different trophic levels can affect each other (Box 6.2).

Box 6.2 | Of mice, moths, and acorns

The gypsy moth and white-footed mouse and the oak trees they both depend on provide an excellent example of how interconnections within food webs can have far-reaching effects (Elkinton *et al.*, 1996). The gypsy moth was introduced from Europe to a Boston suburb in 1869. Gypsy moth caterpillars prefer oaks (*Quercus* spp.) but will eat the leaves of many species of trees during early spring, killing some trees when populations are high but, more often, decreasing tree growth and causing a major nuisance to humans when caterpillars are abundant. After it was first introduced, gypsy moth slowly began to increase in abundance and spread. The spread by this species has been rather slow because females are flightless so this species cannot move very fast on its own. In addition, massive federal programs have been aimed at stopping or slowing the spread.



The generalized relations between the level of acorn production by oak trees, *Quercus* spp. (Year 1) and subsequent white-footed mouse, *Peromyscus leucopus* (Year 2), and gypsy moth, *Lymantria dispar* (Year 3), population densities.

Gypsy moth is an outbreak species, with populations climbing to huge numbers at seemingly erratic intervals and then decreasing to virtually undetectable levels for long periods of time. Outbreaks can be localized but have also been known to extend over large areas. In the USA, population outbreaks have sometimes been extremely damaging; in 1981, gypsy moth populations increased to defoliate 13 million acres (5.3 million hectares) in the northeastern USA. Many times, diseases have been linked with the abrupt declines in gypsy moth outbreaks. The big question has been how outbreaks get started. If we know that, perhaps we can prevent outbreaks from occurring. For many years, scientists and land managers studied factors that might change to allow gypsy moth to increase from low densities and would result in such unchecked population growth. The answer was not readily evident because these population eruptions were actually driven by factors affecting other trophic levels.

Researchers knew that white-footed mice were important predators that eat gypsy moth pupae occurring near the ground. However, acorns produced by oak trees are a dominant food for these mice over the winter. Factors determining the abundance of acorns produced in any one year are complex, including both genetics and weather. Overall, oak trees produce large crops of acorns only every 2–5 years. During the years that many acorns are produced, abundant food is available for the mice and more mice survive the winter. The year after a fall with a great abundance of acorns, the mouse population will have increased and predation on gypsy moth pupae is high. When fewer gypsy moth pupae survive, fewer moths emerge and fewer gypsy moth eggs are laid. The gypsy moth eggs then overwinter (there is only one generation of gypsy moth per year) and the following year there are few gypsy moth caterpillars. Alternatively, when few acorns are produced, the next year, mouse populations will be low and few gypsy moth pupae will be eaten so lots of gypsy moth females will survive to lay eggs. Thus, the third year after a low acorn crop, gypsy moth populations will increase. With the high fecundity of gypsy moth, it does not take many years of decreased pupal predation by mice before gypsy moth populations begin climbing to outbreak numbers. While other natural enemies of gypsy moth certainly play a part, it seems that predation by mice is a key factor keeping gypsy moth populations at low densities. Therefore, those factors affecting mouse populations are indirectly setting the stage for outbreaks of gypsy moth to occur.

FURTHER READING

- Crawley, M. J. (ed.). *Natural Enemies: The Population Biology of Predators, Parasites and Diseases*. Oxford, UK: Blackwell Scientific Publications, 1992.
- Dempster, J. P. & McLean, I. F. G. (eds.). *Insect Populations: In Theory and In Practice*. Dordrecht, NL: Kluwer Academic Publishers, 1998.
- Hawkins, B. A. & Cornell, H. V. (eds.). *Theoretical Approaches to Biological Control*. Cambridge, UK: Cambridge University Press, 1999.
- Huffaker, C. B. & Gutierrez, A. P. (eds.). *Ecological Entomology*. New York: John Wiley & Sons, 1999.
- Krebs, C. J. *Ecology: The Experimental Analysis of Distribution and Abundance*, 5th edn. San Francisco: Benjamin Cummings, 2001.
- Price, P. *Insect Ecology*, 3rd edn. New York: John Wiley & Sons, 1997.

Predators

Use of invertebrate versus vertebrate predators has been strikingly different and these predators themselves have very different attributes. Therefore, these different types of predators will be discussed separately.

7.1 | Vertebrate predators

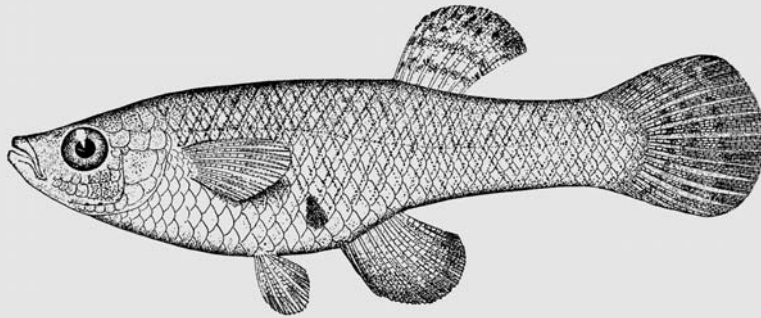
Vertebrate predators are better known to the general public than most invertebrate predators. However, the days for use of vertebrates for biological control are largely over; the prey of vertebrate predators is too unpredictable. Vertebrates are more complex and have a more varied repertoire of behaviors than invertebrates. They can learn in a new environment and switch to new types of prey. However, exactly because vertebrate predators were larger and more obvious, they were used for early biological control introductions. For example, as long ago as 1762, mynah birds, *Acridotheres tristis*, from India were introduced against red locusts, *Nomadacris septemfasciata*, on Mauritius, an island in the Indian Ocean. In 1872, the small Indian mongoose, *Herpestes javanicus*, was introduced from India to Trinidad to control rats in sugar cane. The activity of these voracious predators was said to prevent UK£45,000 of losses in sugar production, an enormous sum at that time. Unfortunately, this early introduction went on to demonstrate the potential problems of introducing vertebrates. The mongoose was predominantly active during the days and rats were active at night. The mongoose became a pest after they quickly learned to kill chickens and the native ground-dwelling lizards and ground-nesting birds. In another disastrous introduction, the cane toad, *Bufo marinus*, was introduced from northern South America into the Caribbean and then Australia to control scarab larvae infesting sugar cane. As with the previous examples, the biology and behavior of this predator were not well enough understood before release and unforeseen side-effects ensued (see Chapter 18).

Vertebrate predators are intelligent and can learn about new types of prey fairly quickly. They are also generally quite omnivorous.

Therefore, vertebrate predators will switch the type of prey they eat fairly readily. As the unpredictable nature of vertebrate predators became apparent, their use for biological control largely ended. However, there remains one exceptional type of vertebrate predator that is still used today to some extent: small predaceous fish called *Gambusia* that feed on mosquitoes (Box 7.1).

Box 7.1 | A fishy tale

The principal species of fish that has been exploited for biological control of arthropods is the mosquitofish, *Gambusia affinis*, commonly referred to as *Gambusia*. This is a small (2.5–5 cm; 1–2 inches long) omnivorous species with high reproductive capacity that can live in shallow water and tolerate changes in temperature and salinity and the presence of organic waste (Garcia & Legner, 1999). These fish are native to the southern USA, Mexico, and the Caribbean and were originally introduced from North Carolina to New Jersey in 1905. By 1975, this species had been introduced for mosquito (Culicidae) control in 50 countries around the world, making them the most widely distributed biological control agent by that time.



Female mosquitofish, *Gambusia affinis*. (Jordan & Evermann, 1900.)

Initially, small numbers of *Gambusia* were introduced to locations with the goal that they would increase on their own over time. Mosquito control efforts changed and then mosquitofish were cultured, harvested, and stored over the winter so that inoculative releases would be possible at specific times, such as after rice fields were flooded.

The resulting control of mosquitoes by these fish has been variable. Control is better when there is a limited alternative food supply for the mosquitofish so that they chiefly prey on mosquitoes. Today, concerns have been voiced regarding use of these fish. *Gambusia* can have a direct impact on native fish through predation on fry (young) or an indirect effect because they are good competitors, with the result that more than 30 species of native fish have been adversely affected after *Gambusia* was introduced. In addition, presence of *Gambusia* has been linked to declines in the general aquatic invertebrate fauna. Because *Gambusia* feed on zooplankton, algal blooms can occur after mosquitofish are introduced. While widespread use of *Gambusia* is not encouraged today, augmentation in contained bodies of water in areas where this species already occurs would still seem to be an acceptable and practical application toward mosquito control, especially if alternative control measures would have a harsher impact on the environment.

7.2 | Invertebrate predators

The range of prey that will be attacked by invertebrates is much more predictable than the range of prey attacked by vertebrate predators. These natural enemies have less ability to switch prey because they are less mobile or less able to control their mobility, and are generally more restricted in habitat use, size of prey that can be caught and eaten, and diet breadth.

Insect predators important for biological control have one of two major types of development. More primitive insects, the Hemimetabola, have immature stages called nymphs that are similar in appearance to adults, although adults are reproductively mature and have fully developed wings. This gradual type of development is seen with praying mantids and true bugs. Mites and spiders also have this type of gradual development. Predators are also found among the more evolutionarily advanced groups of insects having immature stages called larvae (singular = larva) that are very different from adults, with an intermediate pupal stage during which an extensive metamorphosis occurs. Predators with complete metamorphosis, the Holometabola, include groups such as ants, flies, and beetles. For the holometabolous strategy, the needs and habitats of immatures and adults can be very different. As a general rule, adults of invertebrate predators are often more mobile and have better vision than immatures. Adults can therefore lay eggs in locations where prey are present and thus have a huge impact on success of their offspring depending on their choice of locations for laying eggs. Frequently, eggs are laid in areas with aggregations of prey so there will be plenty of food when eggs hatch. With hemimetabolous predators, usually both immatures and adults are predatory but with holometabolous species, this is not always the case. For example, while lady beetle adults and immatures are both predatory, larvae of hover flies are voracious predators while adults visit flowers for food.

Invertebrate predators are often not as adept at finding prey as many vertebrates. They locate the general habitat in which prey are usually found using chemical stimuli, including naturally produced plant volatiles. Once in the correct habitat, to find prey, various invertebrate predators use vision, movement, and chemical stimuli requiring contact. In addition, recent studies have shown that wild tobacco plants (*Nicotiana attenuata*) being attacked by leaf-feeding herbivores released additional volatiles. Exposing an important predator in this system, the big-eyed bug *Geocoris pallens*, to these volatiles induced by herbivory resulted in higher predation levels during experiments. The researchers concluded that predators were using the specific volatiles released by the plants that were being eaten to help improve their ability to locate prey (Kessler & Baldwin, 2001).

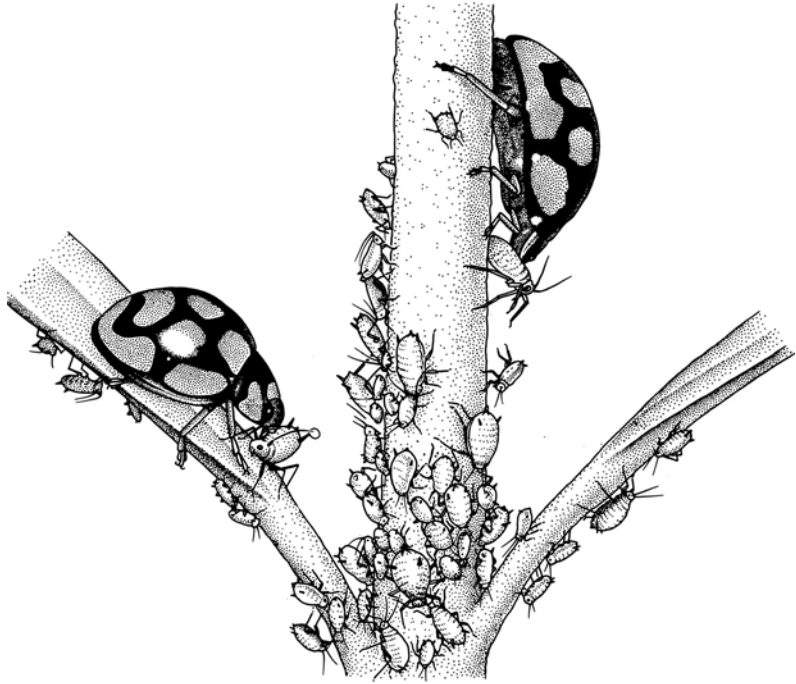
Invertebrate predators utilize a range of methods for capturing prey. In general, the body size of invertebrate predators is larger than that of their prey for species that overwhelm their prey.

Overwhelming prey could be considered the basic strategy of most invertebrate predators. However, some trickier predators do not have to be larger than their prey because they inject poison to kill prey. Others use traps to help them capture prey, for example larvae of ant lions (*Myrmeleontidae*) lie buried in the sandy soil at the bottoms of small pits with their mouths positioned so that any insects falling into the pit will readily be captured and eaten. Ants successfully attack prey in groups, for example army ants marching through tropical rainforests can subdue prey of large sizes due to the sheer numbers of ants simultaneously attacking.

Invertebrate predators actively capture prey using several very different methods. Some mobile predators have good vision, such as ground beetles (*Carabidae*) and jumping spiders (*Salticidae*), and they chase after prey. Others with poor vision use a combination of vision and chemical cues to find prey. For those with very poor vision, such as immature lady beetles, the principal method for detecting prey is tactile, so these predators roam incessantly. Because prey are often aggregated in distribution, as a strategy for finding prey lady beetle larvae first wander in the area of their last meal but, as hunger grows, they roam further and further from their last prey encounter in hope of finding another aggregation of prey. The third major way that invertebrate predators find prey is to sit and wait, often remaining concealed during this time, and then attacking only when prey are present. This “ambush” strategy, well known from the praying mantid, is the best method for catching fast prey, although it requires a lot of patience and a fast response once prey are present. Of course, some predators can be flexible too, integrating the sit-and-wait strategy with active searching.

The flip side to the success of predators finding and catching prey is prey defense. Very mobile prey can simply evade capture, often by running or flying away. Many less-mobile prey have morphological features to deter predation, such as the hard covering of armored scales (*Diaspididae*) or long hairs on tussock moth caterpillars (*Lymantriidae*). Herbivorous insects feeding on some plants can sequester noxious plant compounds. For example, oleander aphids, *Aphis nerii*, sequester toxic cardenolide steroids from host plants and are conspicuously colored yellow and black as a warning. In a study where numerous species of invertebrate predators were fed these aphids, three predator species did not survive, three had decreased growth, and three had the physiological ability to eat this chemically defended prey and develop at a normal rate (Fig. 7.1) (Malcolm, 1992). Therefore, predators can be specialized for overcoming specific prey defenses but not all predators are able to overcome specialized defenses. In a study of predatory ants, which are usually generalists, chemically defended caterpillars were often rejected (Dyer, 1995). The types of defenses employed by pests can definitely have an impact on biological control and can determine which natural enemies will be successful. In an evaluation of classical biological control programs, caterpillars that were visually cryptic (blending in with their

Fig. 7.1 Chemically defended oleander aphids, *Aphis nerii*, cannot be eaten by some predators yet this lady beetle, *Cheilomenes lunata*, has no problems feeding on them. (Illustration by Karina H. McInnes; Gullan and Cranston, 2000.)



surroundings) and had smooth body surfaces had the highest levels of predation and were most successfully controlled by invertebrate predators (Dyer & Gentry, 1999).

Predators ingest prey in different ways. A general insectan model of eating involves use of mandibles for cutting and crushing food with a variety of additional mouthparts assisting in processing a meal (Fig. 7.2). Alternately, some insects such as true bugs have tubular piercing, sucking mouthparts. For predators with piercing-sucking mouthparts, food must be liquid so how do they eat prey? Saliva containing digestive enzymes is injected into the prey and the partially digested prey contents are then ingested. The saliva, in these cases, can also be paralytic or poisonous to arrest movement of prey so that feeding can occur without interruption. Spiders and mites also inject digestive enzymes into prey and then ingest liquefied food.

Predation is a widespread life strategy among invertebrates. Since many predatory invertebrates have life stages with very different morphology and activity, it follows that for some species only certain stages are predatory. Below, we will describe some major groups of predators important in biological control. These will be presented as predators introduced for classical or augmentation biological control and then predators that play important roles in naturally occurring biological control, as part of a community of natural enemies. The predators important in naturally occurring control have been the focus of habitat manipulations in some conservation biological control programs that work to increase populations of predators.

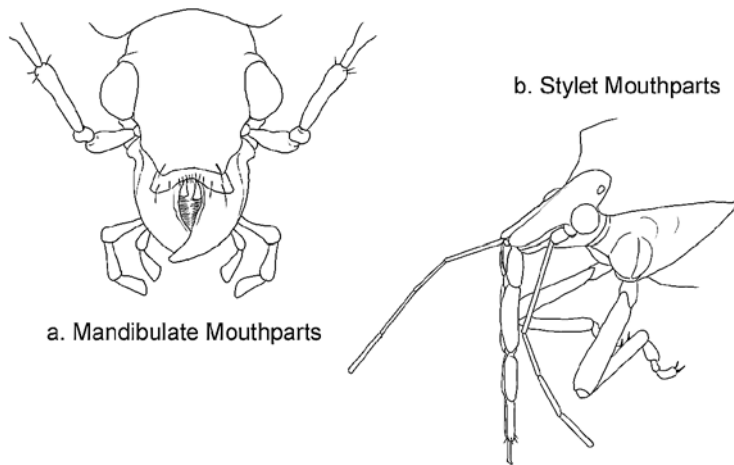


Fig. 7.2 Mandibulate (chewing) versus stylet (piercing–sucking) mouthparts of insect predators. a. Mandibulate mouthparts of the ground beetle (Carabidae) *Calosoma frigidum*. b. Stylet mouthparts of the stink bug (Pentatomidae) *Podisus maculiventris*. Both predators feed on a variety of prey, including gypsy moth caterpillars. (Drawings by A. Burke.)

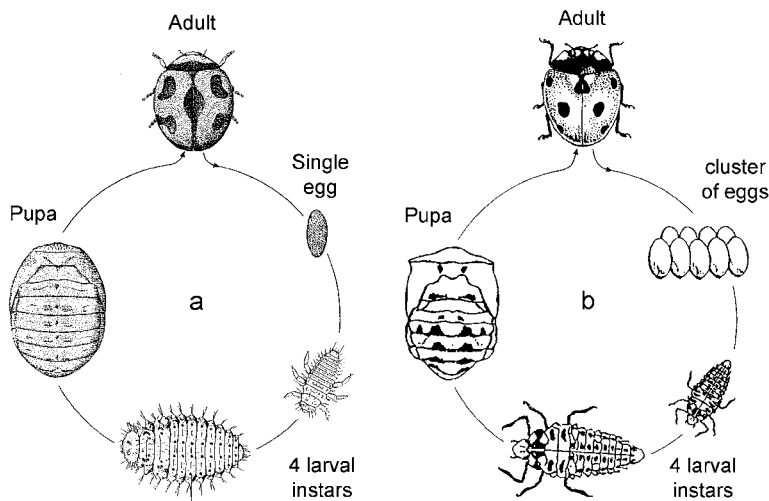


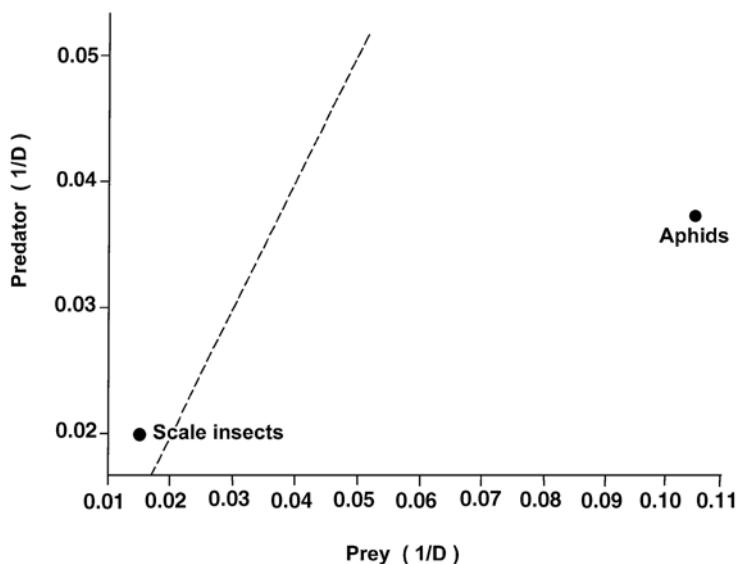
Fig. 7.3 Life cycles of lady beetles demonstrating morphological variability. a. The scale-feeding *Rodolia cardinalis*, the Vedalia beetle (adults 3–4 mm long) and b. The aphid-feeding *Coccinella septempunctata*, the seven-spotted lady beetle (adults 7–8 mm long). (After Dixon, 2000.)

7.2.1 Predators specifically used for biological control

Lady beetles (Order Coleoptera: Family Coccinellidae)

Lady beetles, also called ladybugs or ladybird beetles, are some of the world's experts at eating small, soft-bodied prey such as aphids, whiteflies, mites, mealybugs, and scale insects (Fig. 7.3). Adults of some aphid-feeding lady beetles can consume approximately 100 aphids per day. The well-known adult stages of lady beetles are shiny and convex, with short, clubbed antennae. The family name for lady beetles, Coccinellidae, means clothed in scarlet although many lady beetles are not red and, in fact, many are dull-colored without markings and can be quite small (1 mm long). Patterns on the surfaces of adults help in identifying the species; once patterns on the wing covers (elytra) are formed, they do not change after an adult emerges from pupation and harden.

Fig. 7.4 Comparison of the average developmental rates ($1/D$) of aphid and scale prey and the lady beetle species that feed on them, at 20 °C. The dashed line indicates conditions where the prey and predator develop at the same rate so prey developing slower than predators fall on the left of the line and prey developing faster than predators fall on the right. (After Dixon, 2000.)



The flattened and more elongate immature stages of lady beetles resemble little dinosaurs or alligators more than the adults they will become. Larvae have reduced eyesight and for many species, the larvae must touch their prey with their chemo-sensory mouthparts before they understand it is there. Aphid-feeding lady beetle larvae often hunt by walking quickly, sometimes stopping to swing the front end of their body from side to side, to maximize chances of contacting prey. Once a prey individual is found and has been eaten, then searching in that area becomes more concentrated; this strategy is very well-suited for specializing in prey that occurs in aggregations, such as aphids. Detailed studies have shown that if a larva has recently found and eaten a larger aphid, it will continue searching in an area longer than if it had found only a smaller aphid. To optimize use of aphid colonies, an adult female two-spotted lady beetle (*Adalia bipunctata*) searching for places to lay eggs will leave a colony if she detects the trail pheromone left by larvae of the same species.

Young lady beetle larvae pierce their prey and suck out the contents, while older larvae and adults chew and eat the entire prey. Species of lady beetles often eat only certain types of prey; this has been thought to increase their effectiveness in controlling pests (see Box 3.1). Many species of lady beetles are specialists on either aphids or scales, although a few species eat both. Both aphid- and scale-feeding lady beetles have been used for biological control but classical biological control programs using scale feeders seem to have been more successful than programs using aphid feeders. It has been suggested that this success is tied to the fact that scale-feeders develop faster than their prey (Fig. 7.4) and are more host specific. In contrast, aphids usually develop faster than the aphid-feeding lady beetles and

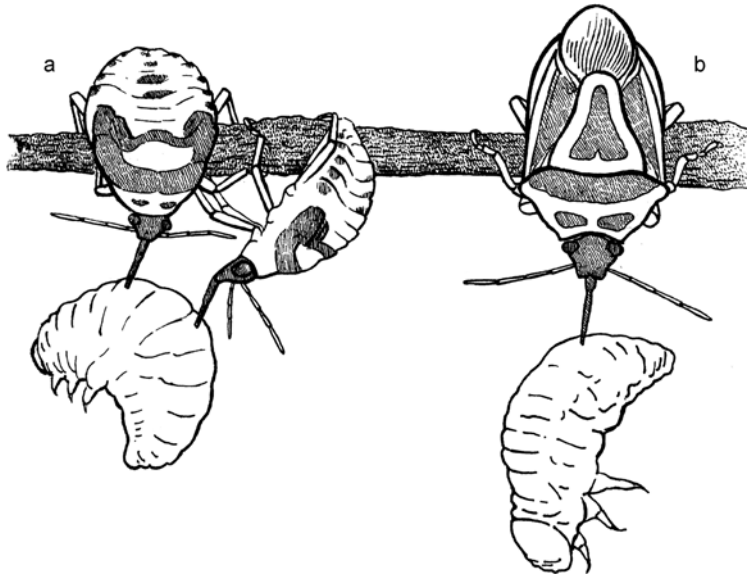


Fig. 7.5 Nymphs (a) and an adult (b) of the stink bug *Perillus bioculatus* feeding on larvae of the Colorado potato beetle, *Leptinotarsa decemlineata*, impaled with their piercing-sucking mouthparts. (Trouvelot, 1931.)

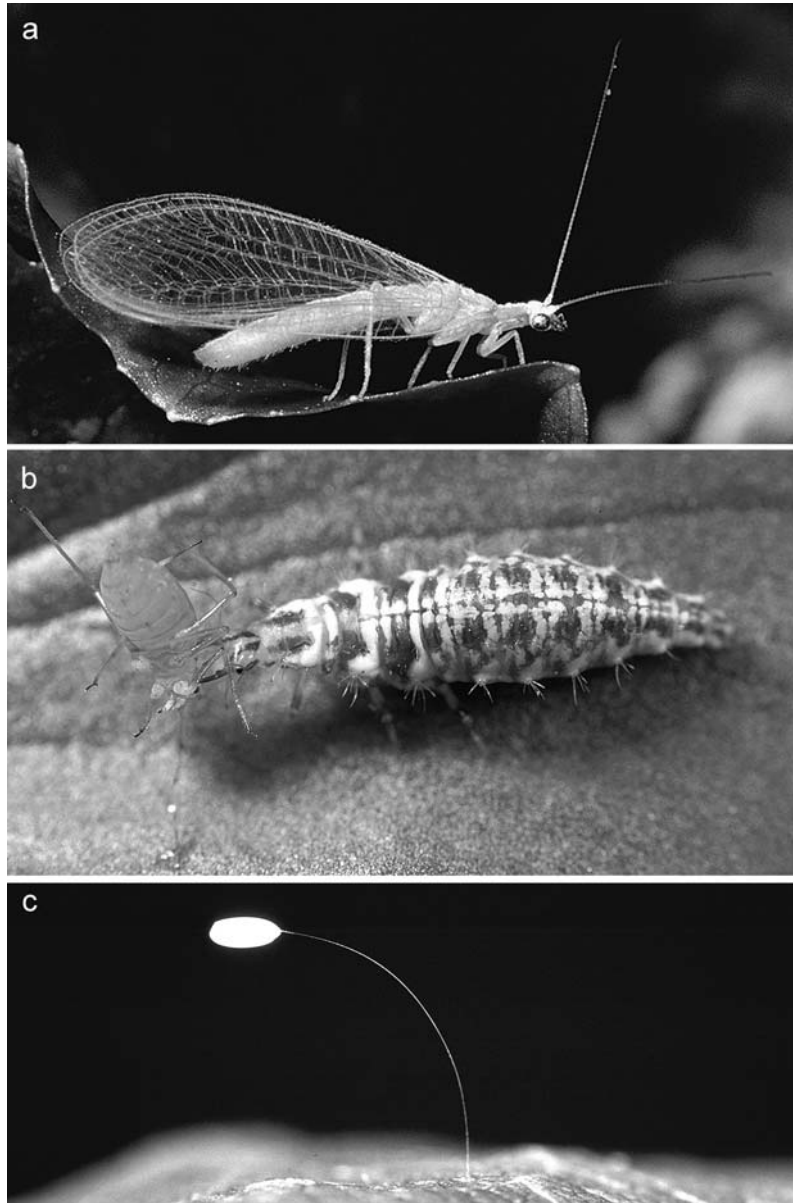
the predators are often unable to keep up with their quickly increasing prey (Dixon, 2000).

True bugs (Order Hemiptera)

Although all insects are commonly called “bugs,” to an entomologist the Hemiptera are the only group of true bugs. Many true bugs are plant feeding but there are also some important predatory bugs. Immature stages of Hemiptera resemble adults, being hemimetabolous, but do not have the fully formed wings. For adults, wings normally are positioned flat, on top of the abdomen. The feature that most differentiates bugs from other insects is their tubular mouthparts. Predaceous species extend their mouthparts forward and use them to spear their prey and to digest prey they inject enzymes, sometimes accompanied by poisons or compounds causing paralysis. Predatory bugs then suck out the body contents of the host (Fig. 7.5). At rest, the mouthparts are held beneath the body and so are not readily visible.

Bugs are often general feeders, both immatures and adults eating eggs, immatures, and adults of a diversity of insects and mites. Stink bugs (Pentatomidae) simply walk toward caterpillars with their mouthparts extended and pierce them. These hemipterans are especially well adapted to feed on prey with lots of defensive hairs or spines because they can eat them while standing a short distance away. Most other terrestrial predatory bugs have some modifications so that legs are used for capturing prey and grasping them while eating. Interestingly, some hemipteran predators, such as the bug *Macrolophus caliginosus* sold for augmentative control of whiteflies in Europe, can facultatively feed on plant materials if prey have all been eaten; their damage to plants is minor and this ability to switch from

Fig. 7.6 The lacewing *Chrysoperla carnea*. a. Adult. b. Voracious larva that often eats aphids. c. Egg. (Photos by Jack Kelly Clark, courtesy University of California Statewide IPM Program.)



predator to herbivore allows these bugs to persist in greenhouses so that they are present if prey populations increase again.

Lacewings (Order Neuroptera)

On spring and summer nights, among the moths at lights you can find green or brown insects with long delicate lacy wings folded tent-like over their abdomens (Fig. 7.6a). Adult lacewings lay their eggs and disperse at night. These adults can be predaceous, some feed on pollen while others do not feed. It is really the larvae of these

holometabolous insects that are the important predatory stage of interest for pest control.

Lacewing larvae are 3–20 mm ($\frac{1}{8}$ – $\frac{4}{5}$ inches) long, with large pointed jaws for skewering their prey. They are perhaps best known for their appetite for aphids and are sometimes called aphid lions, but they will also eat other small insects as well as mites. Lacewing larvae actively search for prey and, once they randomly bump into something, they can identify it as food only after contacting it with their mouthparts. The sickle-shaped mandibles of larvae of both green and brown lacewings are used initially to pierce prey and then digestive salivary secretions are injected (Fig. 7.6b). Ultimately, only the predigested fluids are consumed by the larvae. Because lacewing larvae will eat each other, the adults usually lay eggs at the ends of small stalks so that they dangle in the air (Fig. 7.6c). This prevents the first larva that hatches from eating the nearby eggs of its brothers and sisters.

For biological control, lacewings are released by shaking eggs onto foliage. They have been used in greenhouses and some row crops but to date, releases have not consistently provided control. Lacewings seem particularly vulnerable to predation from other predators (a process termed intraguild predation, see section 7.3.1) and hence may be better-suited for greenhouse releases, where the presence of other predators can be managed. Naturally occurring populations of lacewings are considered important members of resident natural enemy communities.

Predatory mites (Class Arachnida: Order Acarina)

Mites are arthropods, as are insects, but differ from insects in having eight legs, two body parts and no antennae, while insects have six legs, three body parts and antennae. With magnification, one can see that predatory mites are long-legged, and are often pear-shaped and shiny (Fig. 7.7). Mouthparts of predatory mites extend forward from their bodies while mouthparts of plant-feeding mites are directed downwards to the plants on which they feed. Predatory mites use their mouthparts to pierce their prey and inject digestive enzymes. The prey is therefore digested externally and the mite laps up the resulting liquefied mush. Eggs of predatory mites are often quite large relative to the mites and, with magnification, appear round to oblong and pearl-colored. Directly after hatching immature mites (nymphs) have six legs but soon gain another pair of legs. Otherwise, immatures are very similar in appearance to the adults.

Most mites can just barely be seen with the naked eye. Yet, although they are small, pestiferous mites increase in numbers so readily they can create major problems. Predatory mites can be extremely effective natural enemies for control of plant-feeding mites. This has been clearly demonstrated by the ready occurrence of secondary pest outbreaks of mites in agriculture when insecticides kill predatory mites (see Chapter 1). After applications of pesticides, predatory mites

Fig. 7.7 *Hypoaspis* (= *Stratiolaelaps*) *miles*, a soil-dwelling predatory mite used in greenhouses for control of western flower thrip pupae and fungus gnat larvae. (Photo courtesy of David Evans Walter.)



will eventually increase in number again in the sprayed areas since a few will survive the pesticide, or they will recolonize the area through their ability to disperse over longer distances by riding on the wind.

Predatory mites are about the same size as the plant-feeding mites that they attack. Some of the best-known predatory mites are in the Family Phytoseiidae, but predators also occur in many other mite families. Some of these phytoseiids are generalist predators attacking plant-feeding mites as well as insect eggs and small immature insects. *Typhlodromus pyri* is even more of a generalist and may feed on pollen and fungi as well as pestiferous mites, thus persisting well in perennial systems such as orchards, which leads to better biological control (Nyrop *et al.*, 1998). Populations of some predatory mites that readily eat pollen may be maintained in an area by making sure there are pollen sources for times when prey populations are low. Alternatively, some phytoseiids are more specific, preferring pestiferous spider mites and dispersing when prey populations decline. These latter species are known to use chemical cues from the mites themselves as well as host plant cues to locate prey.

Predatory flies (Order Diptera)

Many adult flies are predators and are important members of naturally occurring food webs. However, for biological control, the larval stages of only a few types of flies have received most of the attention; these are the flower or hover flies (Syrphidae) (Fig. 7.8), aphid flies (Chamaemyiidae), and predaceous midges (Cecidomyiidae). While the adults of these groups feed on pollen or nectar, or do not feed, and are excellent fliers, the immatures are predatory maggots without



Fig. 7.8 Syrphid larva feeding on an aphid. The adult is a hover fly, many of which are bee mimics. (Photo by Jack Kelly Clark, courtesy University of California Statewide IPM Program.)

legs. Larvae can move but certainly not fast or far so they really rely on adults to deposit eggs near hosts. Fly maggots have very reduced mouthparts but are still able to hold tightly onto small-bodied prey. Larvae of all of these flies are usually not very host specific, feeding on aphids, mites, scales, and other soft-bodied arthropods that are not very mobile and are smaller than the fly larvae.

7.2.2 Invertebrate predators providing naturally occurring biological control

Praying mantids (Order Mantodea)

Praying mantids can be quite large for insects, often 5–10 cm (2–4 in.) long, and they are probably well known because they are so large. They are called praying mantids because their forelegs are held in an upraised position, similar to the posture assumed for praying by Christians. However, their forelegs are actually held in that position so they are ready to grab prey (Fig. 7.9).

Praying mantids are “sit and wait” predators, often sitting by flowers and eating insects that visit the flowers, including bees, flies, parasitic wasps, and other flower visitors. Therefore, they are effective at catching mobile and not sessile prey, but mantids do not discriminate about what they eat. This extends to eating siblings as they hatch from egg masses. Adult males are even sometimes eaten by females after mating. Because praying mantids are such generalists, they are generally not considered very effective for controlling specific pests.

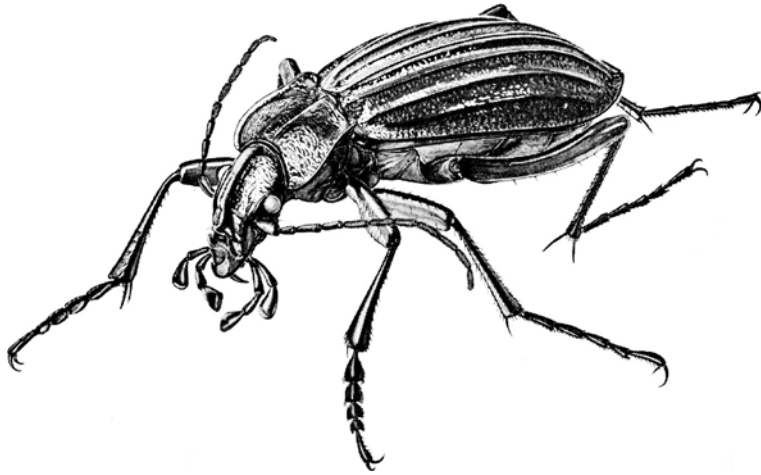
Ground beetles (Order Coleoptera: Family Carabidae)

Ground beetles are the commonly found, dark beetles that hide under stones or in dense plant material on the soil surface. Many are predators although some instead feed on seeds. Adults are medium- to large-sized and are often dark with long legs. They are fast runners and rarely fly (Fig. 7.10). Larvae are elongated and live in litter or soil. Both larvae and adults usually have prominent forward-directed

Fig. 7.9 Praying mantids are some of the largest insect predators. They are ambush predators, remaining motionless while waiting for prey with front legs upraised. (Photo by Jack Kelly Clark, courtesy University of California Statewide IPM Program.)



Fig. 7.10 An adult of the ground beetle *Carabus auronitens*, a brilliant green and golden species (18–26 mm long) common in the forests of central Europe. (Essig, 1942.)



mandibles and actively pursue their prey, especially if it is moving. Adults can cut their prey into pieces with their mandibles and swallow the pieces. Larvae usually use extra-oral digestion; enzymes are introduced into the prey and then the liquefied prey contents are ingested by the larva.

Adults are usually active at night and can eat approximately their weight each day. Most species are generalists and eat a variety of different ground-dwelling prey. A few species climb vegetation to find prey, including *Anchomenus dorsalis* climbing wheat to eat aphids and *Calosoma sycophanta* climbing large tree trunks to eat large gypsy moth

caterpillars. Snail-feeders in the genus *Scaphinotus* are morphologically adapted by having a small head that fits into the opening of a snail shell.

Carabids are important predators providing natural control as part of complex food webs. Their populations can be strongly enhanced by conservation measures and can be seriously reduced when tillage and monoculture leave no persistent habitat for them.

Ants (Order Hymenoptera: Family Formicidae)

Ants are often extremely abundant and successful predators in many types of habitats. In some instances, they are considered the keystone predators in communities. This is true of endemic ants in native ecosystems but can also be seen with invasives. A dramatic example of an ant species that is a keystone predator is the fire ant, introduced to the southeastern USA, where this species has become very abundant and has displaced many endemics.

Ants are related to bees and wasps, differing because they are usually wingless, with the exception of those ants born to mate and disperse. Ants are of course social insects, with two major female castes, the queens and workers, and there are usually morphological subdivisions within the workers.

Many ants are predatory or at least omnivorous, but they are usually generalists in their choice of prey. There has been relatively little research on use of ants for biological control in the USA. While some species are known to be beneficial, the majority are seen as nuisances or even detrimental to biological control. In particular, ants that tend Homoptera (aphids, soft scales, whiteflies, mealybugs) and feed on the honeydew produced, protect these homopterans from predators and parasitoids, so that populations of these pests can increase. Ants also move these honeydew-producing insects from plant to plant. Ants can also disrupt biological control of non-tended pests, such as mites and armored scales, if these occur on the same plants as honeydew-producing species that are being tended.

Spiders (Class Arachnida: Order Araneae)

Spiders are in the same class of arthropods as the mites, also having only two body parts, eight legs and no antennae. Spiders are ubiquitous, are commonly found, and all are predators.

Spiders vary in their behavior, being suited for specific habitats and types of prey. Several of the most common groups will be described. Web builders make many types of webs, but perhaps the best known are the orb weavers (Araneidae) that create lovely spiral orb webs. Orb weavers have poor vision but night or day they are sensitive to vibrations in their webs that potentially signal that struggling prey are caught in the web and cannot escape. Non-web-building spiders include the ground-dwelling wolf spiders (Lycosidae), which wander at night to find prey. During the day, crab spiders (Thomisidae) can often be found on or within flowers, waiting for unsuspecting flying insects to visit the flower for nectar or pollen. Crab spiders can change

color and are often brightly colored so that they match the color of the flower in which they sit. The jumping spiders (Salticidae) have their eight eyes arranged like headlights on their heads to find prey. They wander during the day in the vegetation and on the ground, and can jump impressive distances to reach prey.

7.3 | Specialist versus generalist predators

Many predators feed on a broad range of prey and are then called generalists, or polyphagous. In contrast are the specialists, feeding on one species (monophagous) or only a limited variety (oligophagous) of prey. Actually, it can be difficult to categorize species using these subjective terms. Species that are normally generalists can functionally be specialized in their prey use if they only utilize certain areas inhabited by few prey or if their smaller size restricts them to only the few species of smaller prey in their habitat.

There has been much debate about whether specialists or generalists make better biological control agents. An advantage of generalists is that they can persist in a system when the pest is not present. Therefore, when the pest increases or disperses into the habitat, the predators are already present. There are also some disadvantages to generalists, because they usually do not respond to prey populations in a density-dependent manner and might cause undesired effects if feeding on alternate prey (see Chapter 18). Specialist predators, on the other hand, have the benefit that impacts on non-target organisms are negligible to nonexistent. However, these predators often do not persist as well in an environment once the prey are gone. This can be compensated for, to some extent, if the species readily disperses and would reinvade soon if the pest increased again.

7.3.1 Effects of predators on other natural enemies

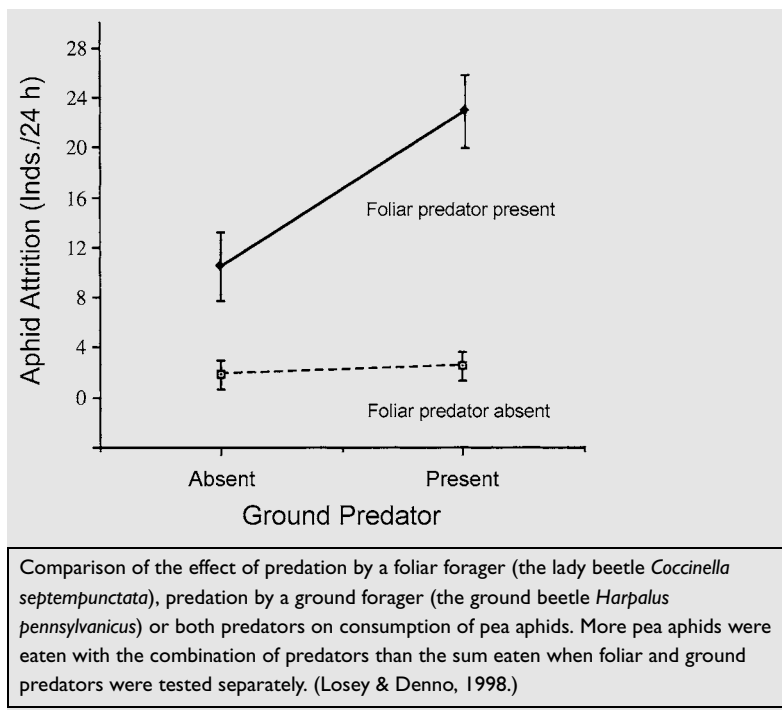
It is important to consider whether predators are generalists or specialists for several practical reasons. Generalist predators, such as praying mantids and spiders, can feed on beneficials, including parasitoids and other predators. Therefore, their usefulness in controlling prey has to be considered relative to the extent to which they influence other natural enemies. In fact, interactions between natural enemies can take several forms: (1) there can be no interaction, (2) natural enemies can kill each other, (3) one natural enemy can interfere with foraging by another, and (4) a natural enemy could influence the behavior of pests making them more likely to be eaten by other natural enemies. Because predators tend more toward being generalists, they have been the focus of interest in such interactions. The principle concerns relative to biological control have been when one predator interferes with the ability of a second to capture prey and, more importantly, when one predator kills another predator, which has been called intraguild predation. Such negative interactions have

been studied in systems with two or more predators known to prey on each other, by testing them singly and then in combinations to evaluate suppression of plant-feeding pests. These studies have clearly shown that adding predators to systems where other predators already occur does not consistently improve pest control; results seem to differ for different systems. Sometimes, there was no change with multiple versus single predators, but in four out of nine examples evaluated the herbivore populations actually increased when multiple predators were present (Rosenheim, 1998), suggesting that intraguild predation had a decidedly negative impact on pest control. In contrast, red imported fire ants (*Solenopsis invicta*) in cotton are voracious predators feeding on herbivores along with other natural enemies. However, while having high populations of fire ants in cotton maximized biological control of most pests, this did not extend to all pests. Fire ants tend cotton aphids, protecting them and feeding on their honeydew, so aphid populations increased to abundant densities because fire ants killed their natural enemies (M. Eubanks, pers. commun.). In contrast, in collards fire ants are not as voracious and, although fire ants eat some parasitized caterpillars, the effects of fire ants and parasitoids are additive and biological control is maximal when both fire ants and parasitoids are abundant.

Few studies have detected a synergistic effect of multiple predators on pests; in such a case, the total effects of two predator species would be greater than adding together the effects of each species alone. However, two predators feeding on the same aphid prey have been shown to have a synergistic effect on predation (Box 7.2).

Box 7.2 | Predators . . . working together?

Aphids and lady beetles have been the subjects of a plethora of studies on predation. Losey and Denno (1998) conducted experiments with two aphid predators simultaneously to ask questions about their interactions. Pea aphids feeding on alfalfa are quite large for aphids, and are very active, readily dropping from plants if disturbed. In individual cages containing alfalfa plants with aphids placidly feeding, either an adult seven-spotted lady beetle or ground beetle *Harpalus pennsylvanicus* or both predators were introduced. After 24 hours, Losey found that aphid populations had declined if the lady beetle alone was present but there was little effect if only the ground beetles were present. In contrast, if both predators were present, more aphids were eaten than if you added the effects of each predator together. What was happening? The lady beetles were disturbing the aphids, which would then drop from plants. Without the ground beetles, the aphids that dropped to the ground simply walked over to the plant stem and climbed back up to the leaves. However, when ground beetles were present, aphids were an unexpected tasty meal that hit the ground. The aphids were easy to catch since they aren't very well adapted to running away from fast-moving larger ground beetles on top of the soil. This study suggests that having both the ground beetles and the lady beetles yields a synergistic effect with more aphids killed than if the effects of each predator alone were added together.



7.4 Use of invertebrate predators for pest control

Predators have been used quite extensively for classical biological control, with increasing emphasis through the years on more host-specific predators. A group that has been used extensively has been the lady beetles (Coccinellidae). This choice is in part due to the early dramatic success with control of cottony cushion scale by a lady beetle (Box 3.1) and, in part, due to the fact that biological control programs have often targeted the introduced aphids and scale insects on which many lady beetles specialize. In recent years, a program against cassava green mite in Africa utilized predatory mites very successfully (Box 7.3).

Predators are also used extensively for augmentative releases. Many small predators are used in greenhouses (Table 7.1). Although many predators are generalists and this can dilute their effectiveness in controlling a specific pest, in the highly controlled greenhouse environment where invertebrate species that are present are usually not desired, being a generalist does not have to be detrimental. Other beneficials, such as parasitic wasps, are released in greenhouses and predation of these natural enemies released to control other pests in the same greenhouse should be avoided. This conflict is usually not a problem because many of the predators that are released prey on pests in different habitats from the pests being attacked by parasitoids; for

Box 7.3 | Mite against mite

The starchy roots of cassava are a major staple food in much of central Africa. For many years after its introduction from South America, this plant had been relatively free of arthropod pests because it possesses high levels of poisonous cyanogenic glycosides and latex that deterred the native African phytophagous arthropods. Cassava green mite, *Mononychellus tanajoa*, was first found on cassava in east Africa in the early 1970s and this pest spread across the cassava-growing region causing up to 80% reduction in cassava root yield.

A classical biological control program was undertaken and the first challenge was obtaining a species name for the mite that occurred in Africa and being able to recognize this same species when collecting in South America, where scientists assumed it had originated. After identification of the cassava green mite, exploration for predatory mites began. Between 1984 and 1988, more than 5.2 million predatory mites from Colombia, belonging to seven species, were imported into Africa and released but none of these species became established. Scientists hypothesized that problems were due to low relative humidity in the cassava-growing area of Africa compared with Colombia, as well as lack of adequate alternative food sources for these predators when cassava green mite populations were low.

In 1988, three species of predatory mites were collected in northeastern Brazil, an area drier than the previous collection areas, and shipped to Africa. Of these three species, clearly the most successful at providing control has been *Typhlodromalus aripo*. This was a big surprise because scientists had initially considered *T. aripo* the least likely to succeed of the three because it seemed less voracious and increased at a slower rate. However, this species turned out to establish, disperse, and persist better than the other two species owing, in part, to its specialized behavior. *T. aripo* lives in the growing tips of cassava plants during the day and forages on the leaves at night (Yaninek & Hanna, 2003). The other two species lived only on the leaves. Mites like humid conditions and in the low relative humidities in the infested areas in Africa, this environmental factor played an important part. Scientists hypothesize that it gave *T. aripo* an advantage to inhabit protected locations during the driest times of the day, compared with the other two species that were not in protected microhabitats. In addition, *T. aripo* is able to persist at low prey densities because it is more of a generalist and will eat pollen and plant exudates as well as cassava green mites.

example, predators feeding on pests in the soil would not impact parasitoids attacking foliage-feeding pests. Pest managers also have control over which species of natural enemies are released and when they are released, and can thus avoid intraguild predation.

What about inundative or inoculative releases of predators in other than controlled environments? In central China, the seven-spotted lady beetle, *Coccinella septempunctata*, has been used extensively to control cotton aphids. This lady beetle is abundant in wheat in May so it is collected and then released in fields of young cotton plants where it suppresses aphid populations early in the season before they have a chance to increase. Releases of predatory mites against plant-feeding mites in orchards in California have also been very successful.

Table 7.1 Common predatory arthropods used for inoculative or inundative releases

Order	Species	Active stages ¹	Use for control ²	Use ³
True bugs (Hemiptera)	<i>Orius insidiosus</i>	N, A	Thrips	I/O
Beetles (Coleoptera)	<i>Cryptolaemus montrouzieri</i> (lady beetle)	L, A	Mealybugs	I
	<i>Carcinops pumilo</i> (hister beetle)	L, A	Fly larvae	I (poultry facility)
Lacewings (Neuroptera)	<i>Chrysopa rufilabris</i> (green lacewing)	L	Aphids	I/O
Flies (Diptera)	<i>Aphidoletes aphidimyza</i> (midge)	L	Aphids	I/O
Mites (Acarina)	<i>Hypoaspis miles</i>	N, A	Thrips, fungus gnats	I
	<i>Mesoseiulus longipes</i>	N, A	Spider mites	I/O
	<i>Neoseiulus californicus</i>	N, A	Spider mites	I/O
	<i>Neoseiulus cucumeris</i>	N, A	Thrips	I
	<i>Neoseiulus fallacis</i>	N, A	Spider mites	I/O
	<i>Phytoseiulus persimilis</i>	N, A	Spider mites	I/O

¹N = nymph, L = larvae, A = adult.

²Many of these predators will feed on numerous types of prey but they are listed here for the prey they are usually released specifically to control.

³I = Indoors, referring to greenhouses, interior landscapes or facilities for raising poultry or livestock;
O = Outdoors, referring to crops and gardens.

C. Glenister, IPM Laboratories, pers. commun.

In particular, use of strains of predatory mites resistant to insecticides proved successful in crops where multiple pests needed to be controlled and insecticide applications were unavoidable. By using strains of predators selected for pesticide resistance, natural enemies were not killed when insecticides were applied (Hoy, 1985).

It is often a little more difficult to document efficacy with larger invertebrate generalist predators than smaller more specialized invertebrate predators. One example would be release of praying mantids in gardens. Although pleasant to maintain as residents, praying mantids are often not efficacious for pest control. Convergent lady beetles provide another example of a predator that does not control garden pests after release (Box 7.4). Release of insectary-reared lacewings to control grape leafhoppers (*Erythroneura* spp.) in vineyards has also not been very consistent, although lacewings are known to be voracious predators. Several avenues for improving efficacy have been proposed including choosing the correct lacewing strain and further adjusting numbers and methods for release.

Conservation methods rely on naturally occurring predators whose populations can be conserved or enhanced. In this way,

Box 7.4 | *Hippodamia convergens* takes wing

This species is named convergent because it has two converging white lines on its black thorax, forward of its black-spotted orangish wing covers (elytra). These beetles prefer to feed on aphids, but aren't so choosy about where the aphids are living, ranging from gardens to trees to field crops. Convergent lady beetles are native to the western United States, where their specialized behavior pre-adapted them to be used for biological control. In summer, when aphid populations decline in the California Central Valley, these beetles fly to higher elevations in the foothills of the tall Sierra Nevada Mountains to the east. In the mountains, the beetles feed on pollen and nectar and then eventually aggregate in large numbers in canyons where they spend the winter. In early spring, when temperatures begin to warm and reach 18 °C (65 °F), adults mate and fly up to catch the winds that carry them to the floor of the Central Valley where they feed and reproduce.

Many years ago, people found the large aggregations of adult beetles in the mountains and decided this seemed like a nice way to provide agents easily for biological control of aphids. It was fairly simple to find aggregations, collect large numbers of beetles and then keep the beetles cool until they were purchased and released. The only problem is that whenever and wherever the beetles are released, they think that it is early spring and that they are in the mountains and it is time to go to the Central Valley. So, after release, the vast majority of beetles fly up and away. Researchers have conducted studies to see if they could find ways to manipulate the beetles so that they would remain where they have been released. However, due to this innate dispersal behavior, these beetles have never been successful for large-scale releases in field crops. In greenhouses and on small plants in specific areas, releasing large numbers of beetles can reduce aphid numbers temporarily. It seems that wetting plants and releasing beetles under plant stems or trunks in the evening may help to slow dispersal. This example demonstrates that some of these natural enemies have specific ingrained behaviors and in some instances, the behaviors of natural enemies must be considered as it will affect their use for biological control.

predators that would be far too difficult or expensive to mass-produce, even if techniques had been developed, can be used for control.

FURTHER READING

- Brandmayr, P., Lövei, G. L., Zetto Brandmayr, T., Casale, A. & Vigna Taglianti, A. (eds). *Natural History and Applied Ecology of Carabid Beetles*. Sofia: Pensoft, 2000.
- Coll, M. & Ruberson, J. R. (eds.). *Predatory Heteroptera: Their Ecology and Use in Biological Control*. Lanham, MD: Entomological Society of America, 1998.
- Dixon, A. F. G. *Insect Predator-Prey Dynamics: Ladybird Beetles and Biological Control*. Cambridge: Cambridge University Press, 2000.
- Evans, D. L. & Schmidt, J. O. (eds). *Insect Defenses: Adaptive Mechanisms and Strategies of Prey and Predators*. Albany, NY: State University of New York Press, 1990.
- Hodek, I. & Honek, A. *Ecology of Coccinellidae*. Dordrecht, NL: Kluwer Academic Publishers, 1996.

- Holland, J. M. (ed.). *The Agroecology of Carabid Beetles*. Andover, Hampshire, UK: Intercept, 2002.
- Hoy, M. A., Cunningham, G. L. & Knutson, L. (eds). *Biological Control of Pests by Mites*. Division of Agricultural Sciences, University of California, Publication 3304, 1983.
- McEwen, P., New, T. R. & Whittington, A. E. (eds). *Lacewings in the Crop Environment*. Cambridge: Cambridge University Press, 2001.
- New, T. R. *Insects as Predators*. Kensington, NSW, Australia: New South Wales University Press, 1991.
- Rosenheim, J. A. Higher-order predators and the regulation of insect herbivore populations. *Annual Review of Entomology*, **43** (1998), 421–447.
- Symondson, W. O. C., Sunderland, K. D. & Greenstone, M. H. Can generalist predators be effective biocontrol agents? *Annual Review of Entomology*, **47** (2002), 561–594.

Chapter 8

Insect parasitoids: attack by aliens

In the 1979 movie *Alien*, starring Sigourney Weaver, a crew member traveling through space with Weaver becomes infested with an alien life-form. The alien develops within the crew member until almost the crew member's size and then emerges dramatically from the crew member's chest, killing him as it emerges. This screenplay could have been written by a parasitoid biologist. While the aliens are portrayed as bad and scary in the movie, in nature, parasitoids of insect hosts are part of complex food webs and their use in regulation of insect pest populations is a cornerstone of biological control.

Parasitoids are therefore a second major type of natural enemy used to control invertebrates. Parasitoid is a term derived from the more general term parasite. Parasites are organisms living in or on other organisms, from which they gain nourishment. The term parasitoid specifically refers to insects that parasitize other insects when they are immature but are free-living when adult. Parasitoids can be distinguished within the larger category of parasites because they eventually kill their host after completing development and use only a single host. In contrast, predators usually consume several hosts (prey) to develop. Many parasitoids have a holometabolous life style that allows the different life stages of parasitoids to specialize in different ways at different ages. Immature parasitoids are often soft-bodied, grub-like or maggot-like in form, and remain in close association with hosts to maximize their growth and development. The immatures feed on hosts either externally or internally and usually have no legs or eyes. The free-living adult parasitoids have eyes, antennae to detect chemical cues, legs, and wings. Adults are therefore usually the mobile stage, being better able to disperse, find a mate and find healthy hosts for development of their progeny.

The size of adult parasitoids may be influenced by the size of their hosts. In all cases, the size of the host when it stops growing puts an upper limit on parasitoid size because the host is the sole food source during parasitoid development. Some adult parasitoids, feeding as immatures on large wood wasps within the wood of tree trunks, can reach up to 10 cm in total length. At the other extreme, the fairy flies (Mymaridae), which develop within insect eggs, are among the

smallest multicellular eukaryotes, sometimes 0.2 mm long, smaller than some unicellular Protozoa! Due to their close physiological association with their hosts, many parasitoids are quite host specific, able only to develop in one stage of one or more host species.

8.1 | Taxonomic diversity in parasitoids

Although the parasitoid life strategy seems rather specialized, it is a life history strategy that has been exploited by numerous groups of insects. Parasitoids are extremely common among wasps (order Hymenoptera), less common among flies (Diptera) and, although found in the beetles (Coleoptera), moths and butterflies (Lepidoptera), and lacewing order (Neuroptera), this life style is rare in these latter groups.

8.1.1 Parasitic wasps (Order Hymenoptera)

It has been estimated that there are more than 65,000 species of Hymenoptera that develop as parasitoids and many of these species have not been described (Gordh *et al.*, 1999). The largest and most noticeable parasitoids generally belong to the Ichneumonoidea, the superfamily that includes the Ichneumonidae and Braconidae (Fig. 8.1a). The superfamilies Chalcidoidea and Proctotrupoidea are other very diverse groups, but are much less noticeable with many species only a few millimeters long (Fig. 8.1b, c).

Once mated, females of parasitic Hymenoptera can control fertilization of their eggs. Since males develop from unfertilized eggs, they can therefore control the relative numbers of males and females according to whether eggs are fertilized or not. Males in many species are smaller than females and adult females are known to lay male eggs in smaller hosts that would only support development of a smaller and probably less fecund female.

Females have an elongated tubular egg-laying structure, called an ovipositor. In some parasitoids, this structure extends far beyond the body of females and can be very conspicuous. In the bees and social wasps, all of which are related to parasitoids, the ovipositor has evolved for use as a sting for defense. Adult female parasitoid wasps instead use the ovipositor to inject eggs into hosts or lay eggs on top of hosts (Fig. 8.2). Using an ovipositor, parasitoid wasps can be precise about depositing their offspring where they have the best chance of survival. In parasitic Hymenoptera, the length of the ovipositor often reflects the type of host that is parasitized and where that host lives. For species laying eggs within hosts, the ovipositor is adapted to pierce the host cuticle and an egg then passes down the ovipositor and is deposited within the host. Other parasitic wasps lay eggs on top of host larvae, and often these hosts live within a concealed location such as a cocoon, a leaf mine, a plant gall, or even within the wood of a tree. In these instances, the ovipositor is used to drill through the material, often part of a plant, surrounding the host. How can

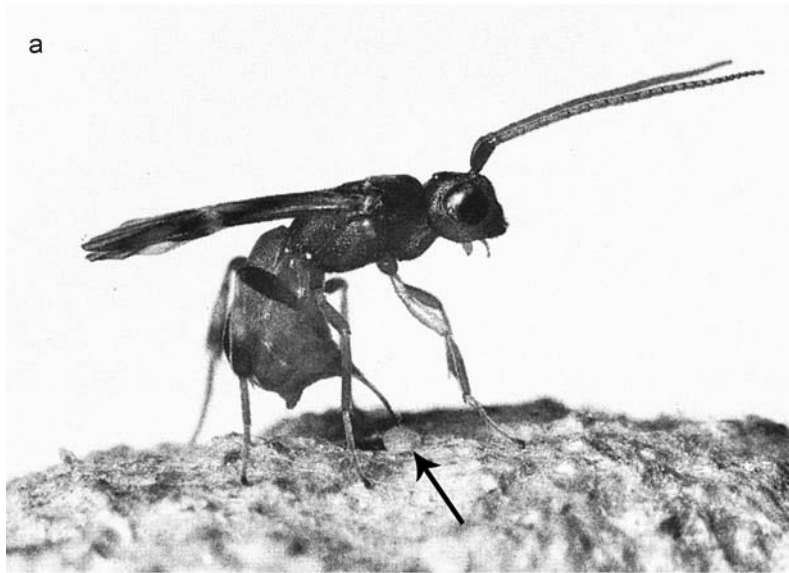
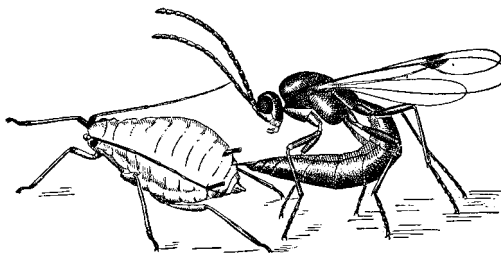


Fig. 8.1 a. The braconid parasitoid *Phanerotoma flavitestacea* (Ichneumonoidea) laying an egg in an egg of its host, the navel orangeworm, *Amyelois transitella*. The larva of this egg-larval parasitoid develops in the host larva and pupates after the host spins a cocoon to pupate. (Caltagirone et al., 1964.)

b. Tussock moth parasitoid *Spilochalcis* sp. (Chalcidoidea: Family Chalcididae). c. The tiny *Anaphes iole* (c. 0.5-0.6 mm long) laying an egg within an egg of lygus bug (Chalcidoidea: Mymaridae). (Photos b and c by Jack Kelly Clark, courtesy University of California Statewide IPM Program.)



Fig. 8.2 Braconid parasitoid ovipositing in the aphid *Schizaphis graminum*. The aphid is approximately 2.2 mm long. (Webster, 1909.)



the wasp, on the outside of the plant, determine that there is a larva within, exactly where the host is located or even when the ovipositor reaches the correct location during drilling? Antennae are used to locate the general area but the tips of the ovipositor and the ovipositor sheath contain different types of sensory cells that are thought to detect both mechanical and chemical stimuli, thus providing additional information for the probing female parasitoid.

8.1.2 Parasitic flies (Diptera)

Flies are second only to the wasps in developing different strategies enabling life as a parasitoid (Fig. 8.3). Most flies do not have the advantages of a piercing ovipositor for injecting eggs into hosts and they may be less precise when depositing their young. Parasitic flies mostly attack exposed hosts and not hosts living in concealed locations within plants or plant galls. However, there is still diversity among the strategies used by parasitic flies for depositing young where they will successfully be able to parasitize hosts. Some species



Fig. 8.3 Tachinid fly *Eucelatoria armigera*, whose larvae develop within bollworm larvae, *Helicoverpa zea*. (van den Bosch & Hagen, 1966.)

glue their eggs externally on hosts and, after hatching, the larvae pierce the host cuticle and enter the host's body where they grow and develop. Other parasitoid flies produce many tiny (0.2 mm) eggs, called microtype eggs, that are laid on foliage. The lucky eggs are then eaten by hosts after which they hatch in the host gut and begin to develop within the host. The family Tachinidae is perhaps the most diverse family of parasitoid flies, with over 8,000 species. Adults of this group can easily be mistaken for large houseflies although their life cycle is certainly much more complex, requiring a living host for development.

8.1.3 Parasitic beetles (Coleoptera)

Beetle parasitoids are less diverse, but the very different method for finding hosts employed by some species is worth describing. Although the immature stages of most parasitoids are not mobile, in some parasitic beetle species, the very small first instar has legs and is very mobile (a triungulin form). Some triungulins are adapted for attaching to adult bees so they are subsequently transported to bee nests where they locate and parasitize the bee's offspring. Subsequent instars are legless, and thus remain living in or on their food supply. For such a species, eggs can be laid away from hosts, do not require food and the mobile first instars are attracted to moving objects. As might be expected, species with these mobile first instars lay many eggs due to the low chances of both finding and attaching to hosts.

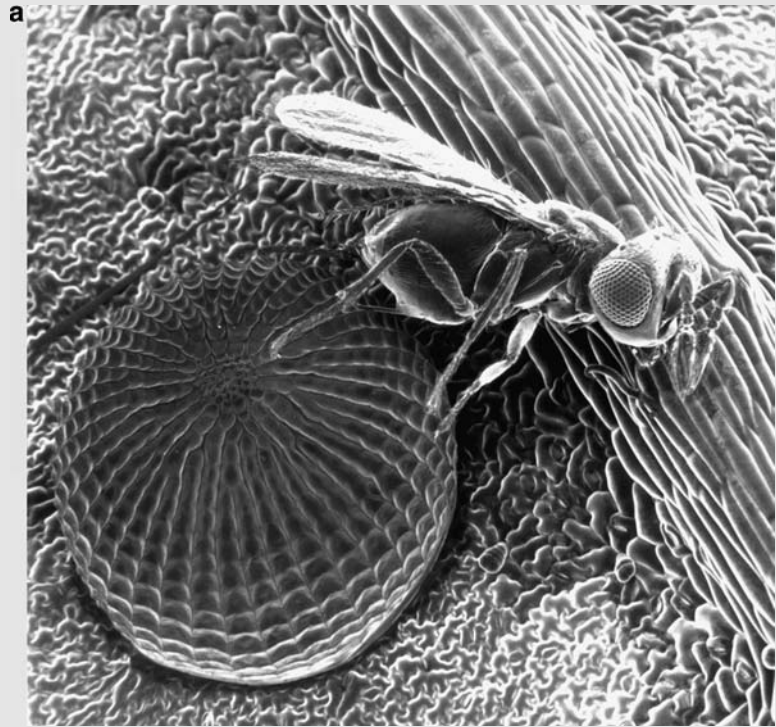
8.2 Diversity in parasitoid life histories

Life history strategies among parasitoids are extremely diverse and can be quite intricate, often with finely tuned associations between parasitoids and hosts. Parasitoids most often develop on immature stages, such as eggs, larvae or pupae, although occasionally adults are hosts. Individual species of parasitoids are usually highly specialized regarding the host stage attacked. Some very small species of parasitic wasps, such as tiny *Trichogramma*, attack host eggs (Box 8.1), while larger parasitoids develop within later instars, pupae, and adult hosts. For some parasitoids, eggs are injected into early instar larvae but parasitoid larvae do not develop until the host has reached a later developmental stage. For example, “egg-larval” parasitoids lay their eggs within host eggs but the parasitoid larvae do not develop until hosts become larvae.

Box 8.1 Finding the right egg

Trichogramma are among the smallest parasitic wasps (0.2–1.5 mm) but have been the subjects of more studies than any other parasitoids. These egg parasitoids have short ovipositors and, being members of the Chalcidoidea, they have relatively short, elbowed antennae. Members of this genus are solitary or gregarious

endoparasitoids, developing within the eggs of a broad range of hosts including many crop pests. Being very small and therefore not strong fliers, *Trichogramma* are often more habitat specific than host specific. A single species can parasitize the eggs of a number of different host species but the resulting adults will vary in size based on the size of the host egg. When the female wasp emerges from a host egg in which she developed, all of her own eggs are fully developed (proovigenic). In fact, as with all parasitic Hymenoptera, *Trichogramma* do not have to find mates to begin laying eggs. However, without fertilization, eggs develop but will all become males. Only fertilized eggs will become female.

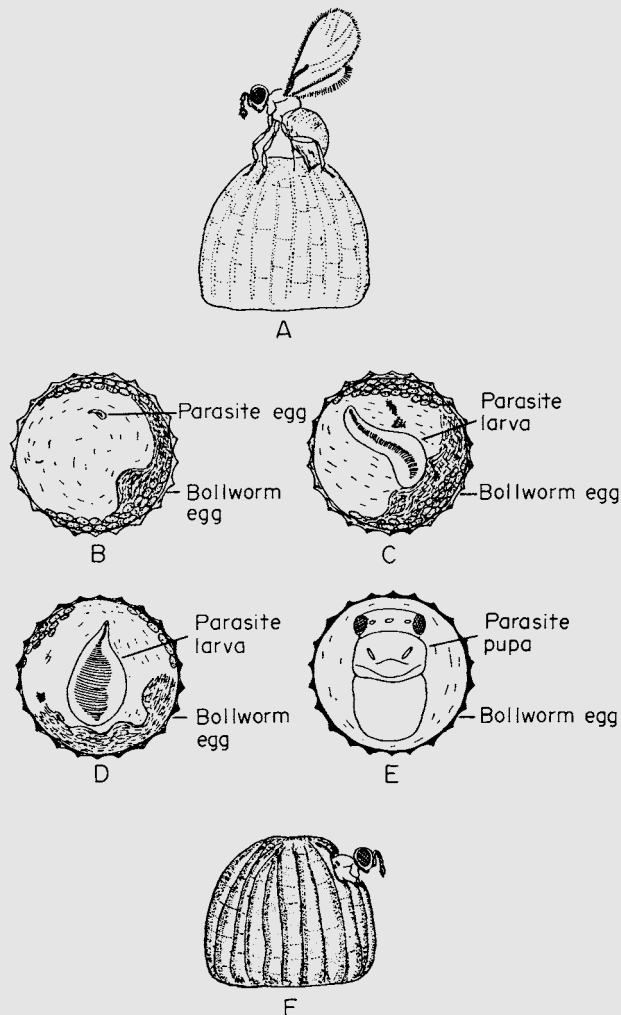


The chalcidoid egg endoparasitoid *Trichogramma*. a. Adult female ovipositing in cabbage looper, *Trichoplusia ni*, egg (Photo courtesy of G. Carner). b. Life history of *Trichogramma*. (A) Female ovipositing in bollworm egg, (B) *Trichogramma* egg within bollworm egg, (C, D) Parasitoid larva developing, (E) Parasitoid pupates within the host egg shell, (F) Adult wasp emerges from the egg. (From van den Bosch & Hagen, 1966.)

Detailed studies have been conducted on recognition and acceptance of host eggs by adult female *Trichogramma* (Schmidt, 1992). *Trichogramma* look for small, rounded objects and will even attempt to lay eggs within glass beads of the correct size. After finding a host egg, a *Trichogramma* female examines the host surface, walking back and forth on it and drumming with her antennae for 10–40 seconds, with the length of examination based on the curvature of the egg surface. The female examines the egg for so long with good reason. She can detect marker chemicals deposited when other *Trichogramma* have been walking on the egg and

she wants to avoid laying her eggs in previously parasitized host eggs, if possible. The external marker is water soluble so what if it has rained? The female begins drilling with her ovipositor and, with some experience, she can detect whether parasitoid eggs already occur within the egg or not. But host eggs can be difficult to find and females get frustrated. If a female does not find unparasitized host eggs within 10 minutes she will keep looking but after 90 non-productive minutes, *Trichogramma* females give up and will lay eggs within a host egg regardless of previous parasitization. The number of eggs laid is regulated by the size of the host egg, ranging from 1 to 4 *Trichogramma* eggs per host egg.

b



Trichogramma adults are winged but, being so small, they are not very capable of controlling where they go. Host eggs are often aggregated so the *Trichogramma* just needs to find oviposition sites. The clever adult wasps can attach to mobile adults of hosts and hitch a ride, only getting off once the host begins to lay eggs. This works well because the host takes the parasitoid to the oviposition site and then the parasitoid is ensured of locating eggs that are freshly laid.

Table 8.1 Generalized life history strategies of koinobionts and idiobionts

	Koinobiont	Idiobiont
Location for development of parasitoid relative to host	Endoparasitic	Ectoparasitic
Host development after parasitoid oviposition	Continues	Ceases
Location of host	Exposed	Concealed
Host specificity	Specialists	Generalists

From Quicke, 1997.

Parasitoids having hosts that continue to develop after oviposition are called koinobionts (*koino*="shared," *biont* = "life"). Alternatively, those species for which the host is killed or paralyzed after oviposition are called idiobionts (*idio*="single," *biont* = "life"). Koinobionts lay eggs in the younger, generally more abundant, host stages that may be easier to find, and the hosts continue to grow larger to provide more food for the parasitoid. However, while they develop, the parasitoids must contend with host defenses, and the often-extended immature stage means they are also more likely to fall prey to other natural enemies. For idiobionts, the host is more like a piece of meat; while idiobionts are assured of their food source, the amount of food will not increase, and idiobionts of later stages may have to search longer because many hosts are older and therefore less abundant. As we discuss different aspects of parasitoid life history strategies further, you will see that there tend to be suites of characteristics that often occur together; some of these are listed in Table 8.1.

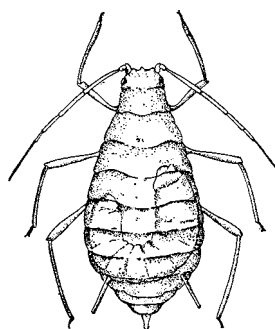


Fig. 8.4 Larva of an aphidiid parasitoid (Ichneumonoidea) developing within the aphid *Schizaphis graminum*. The aphid is approximately 2.2 mm long. (Webster, 1909.)

There are numerous ways that parasitoids develop with respect to their hosts. The most common are the endoparasitoids, which develop within hosts (Fig. 8.4). Endoparasitoids are adapted to living within a mass of semi-liquid food and have very reduced, cylindrical bodies with few sense organs, closed spiracles (openings of the insect respiratory system), limited mobility, and a thin cuticle. Alternatively, some parasitoids lay their eggs on top of hosts; often when these larvae hatch from eggs, they attach to the host using their mouthparts and then continue development, living as ectoparasitoids (Fig. 8.5). These locations of the developing parasitoid larvae relative to the host often are also associated with the generalized life history strategies (Table 8.1). As a general trend, endoparasitoids are often associated with exposed hosts and hosts continue to develop after oviposition (koinobionts). It is thought that endoparasitoids have more limited host ranges because the immature stages must specialize to evade the immune responses of hosts. Ectoparasitoids are often associated with concealed hosts, such as caterpillars living within fruit, or beetles within wood, where they kill or paralyze their hosts during or soon after oviposition (idiobionts). Thus, ectoparasitoids are more specialized by host location and when adult ectoparasitoids lay eggs they often search for a specific type of habitat and not a specific host. Because they live externally on hosts, evading the host immune sys-

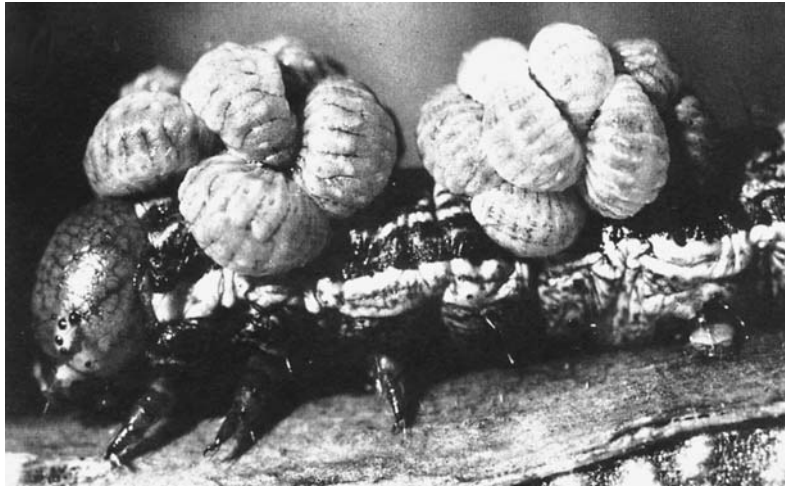


Fig. 8.5 Larvae of the chalcidoid *Euplectrus* sp. (Family Eulophidae), a gregarious larval ectoparasitoid on an armyworm larva. (Photo courtesy of James Carey.)

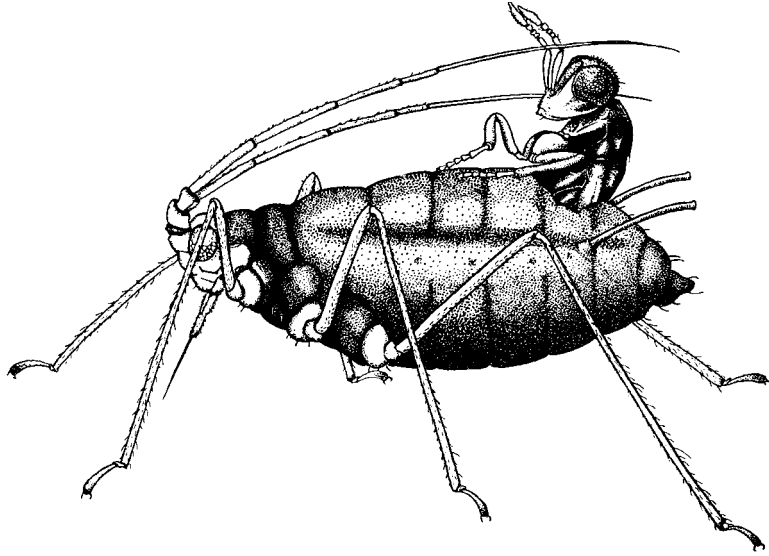
tem is not such a necessity. In fact, it is thought that ectoparasitoids are frequently more general in host range because they are not developing within the body of the host and their growth environment is not as specialized.

After consuming much or all of the host, parasitoids pupate within the hosts' body or exit the host to pupate. Those that leave the host may spin a cocoon in which they pupate on top of or next to the host's body (as in the cover photo). Parasitic wasps remaining within the host's body can use the hardened host cuticle for protection while they pupate. Parasitic flies often create a smooth hardened covering within which they pupate.

Parasitoids also are diverse in whether they share an individual host and how they do this. In solitary parasitoids, one egg is laid and one parasitoid larva develops within one host and emerges (Fig. 8.6). In gregarious parasitoid species, multiple eggs can be laid per host and many individuals can develop within one host. Most amazingly, in polyembryonic species, a single parasitoid egg laid within a host can divide to produce from 2 to more than 3,000 genetically identical individuals, depending on the size of both the host and the parasitoid. We could hypothesize that there are trade-offs in development of a solitary versus polyembryonic type of life; a solitary parasitoid developing within a host larva would produce one adult wasp that would be quite large and better able to control its dispersal, while a polyembryonic species using the same size of host would produce many individuals but the resulting wasps are extremely small and would then have diminished capabilities for having control over their dispersal.

In the case of multiple parasitism, individuals of different species oviposit in or on the same host. In most cases, only one parasitoid species survives to emerge. One species may always be victorious, or either species may win depending on which species laid its egg first. Superparasitism results when more than one egg or clutch of eggs of a particular parasitoid species are laid within one host. Superpara-

Fig. 8.6 The parasitoid *Aphelinus jucundus* (Chalcidoidea) emerging from its aphid host *Acyrtosiphon malvae*. (Griswold, 1929.)

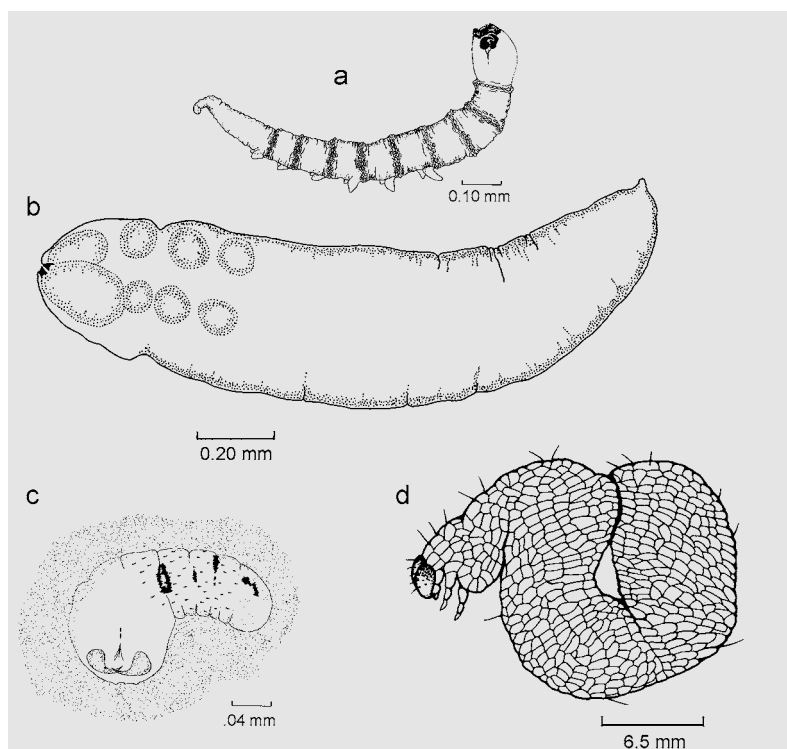


sitism in parasitoids may result in all progeny being reduced in size, or in one female's progeny being killed. Often the offspring of later-arriving females are disadvantaged relative to the earlier female's offspring. Researchers have been very interested in how superparasitism is avoided by adult females. In many examples, this is accomplished by adult females using their ovipositors to deposit a chemical marker on the surface or inside a parasitized host. This marker acts as a deterrent to other parasitoids to avoid superparasitism. When superparasitism or multiple parasitism does occur, larvae of endoparasitoids may fight within hosts and can kill each other by biting. A specialized instance of larval fighting occurs within some polyembryonic species, with non-reproducing, specialized "defender-morph" larvae that act solely in defense of their genetically identical siblings (Box 8.2).

Box 8.2 | A precocious parasitoid

Parasitoid life cycles can be fascinating in their complexity and variability. The life cycle of the parasitic wasp, *Copidosomopsis tanytmenus*, attacking the Mediterranean flour moth (*Ephesia kuehniella*), a stored product pest, provides just such an excellent example of this complexity. An adult female of these tiny wasps (1.26 mm long to the end of the ovipositor) lays an egg within a host egg. This is an egg-larval parasitoid so that the host continues to develop and, in fact, does not die until it would normally spin a cocoon, 6 weeks later. Instead of producing a cocoon, the host larva dies and becomes a mummy, filled with 100–200 pupae of *C. tanytmenus*.

After the parasitoid egg is laid, it begins development as a mass of undifferentiated cells, a polygerm. Each cell will eventually grow into a wasp. The first one or two parasitoid larvae that develop are always precocious and are found in the host 10 days after the parasitoid oviposits, and with time, more precocious

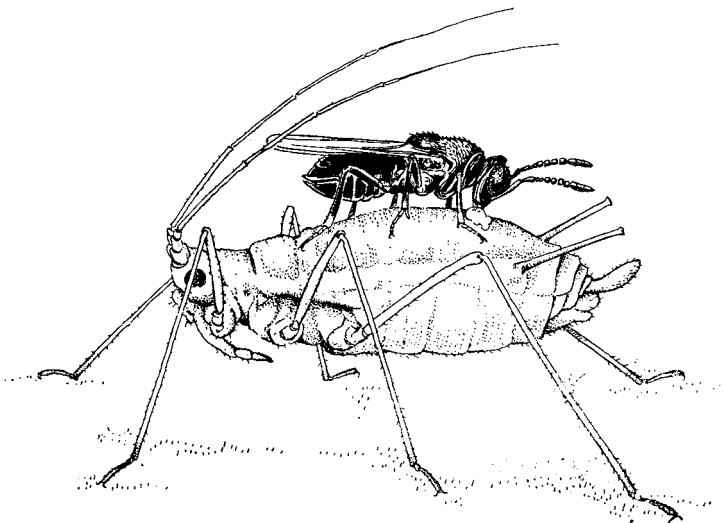


a. A precocious larva of *Copidosomopsis tanytmemus* from a 14-day-old host Mediterranean flour moth caterpillar, *Ephestia kuehniella*. b. One of the 164 mature normal *C. tanytmemus* larvae from a 40-day-old host caterpillar. c. Capsule formed from caterpillar blood cells, containing dead, wounded larva of *Phanerotoma flavitestacea* after this competitor was attacked by the precocious larva of *C. tanytmemus* (Cruz, 1981). d. Cadaver of a larva of the silver-y moth, *Autographa gamma*, filled with cocoons of the polyembryonic parasitoid *Copidosoma truncatellum*. (From Silvestri, 1906.)

larvae can be found (Cruz, 1981). The asexual precocious larvae are thin with large mandibles and, as the polygerm increases in size, these first emergers, the so-called precocious larvae, play the role of defenders. If another parasitoid lays an egg within the host, once that egg begins to develop, the invader is attacked by a precocious larva. The wounded invader is then recognized as non-self by the host and is encapsulated by the immune system. By 4.5 weeks after oviposition, the individual cells of the polygerm differentiate into normal parasitoid larvae that are sac-like with only very small mandibles. These normal larvae grow quickly and are mature by the sixth week. The initial defenders, the precocious larvae, do not develop further and are all dead by week 6. Thus, the precocious larvae prevent superparasitism as they develop but they are altruistic, defending their sisters and brothers but not surviving to reproduce.

Of course, the sizes of parasitoids are influenced by the sizes of hosts and how hosts are utilized. As one can imagine, the largest parasitoids are all solitary with larger hosts, while the smallest parasitoids are gregarious, polyembryonic, or develop within host eggs.

Fig. 8.7 The hyperparasitoid *Aphidencyrthus aphidivorus* (Chalcidoidea) feeding at an ovipositional wound in its aphid host, *Acyrtosiphon malvae*. (Griswold, 1929.)



While most parasitoids attack insects feeding on plants or other non-insect resources in the environment (primary parasitoids), some parasitoids have taken this one step further and attack the parasitoids developing within previously parasitized hosts (these are called hyperparasites or hyperparasitoids). An extreme case of hyperparasitism has been found with species for which males can only develop by feeding on females of their own species living within parasitized hosts. Hyperparasites can at times be extremely abundant and in some cases their activity has jeopardized the effectiveness of primary parasitoids being released for biological control.

Not only the immatures of parasitoids require food. Many adult parasitoids live longer if they feed and food certainly also provides more energy for the extensive searches required to find low-density hosts. Foods for adult parasitoids are often nectar, honeydew, and pollen. A behavior called host feeding also occurs in species within 17 families of parasitic wasps. The adult female wasp usually creates a hole in the host body wall using her ovipositor and then turns around to eat the exposed host blood and sometimes tissues too (Fig. 8.7). This can occur after a parasitoid egg has been laid and, in these instances, feeding by the parasitoid does not kill the host so that the wasp progeny can successfully develop. More frequently, host feeding occurs when no egg is laid and then host feeding by the wasp usually kills the host. Host feeding is characteristic of parasitoid species that need food as adults to produce eggs that contain an abundance of yolk or species with adults that mature their eggs throughout their lives (synovigenic). Adult parasitoids that emerge with their full complement of eggs (proovigenic) still require sugar for energy but do not require the lipid-rich nutrients that they would get from feeding on hosts. Idiobionts are often synovigenic while koinobionts are often proovigenic. Parasitoids that host feed may thus kill hosts when acting as predators as well as through parasitism. For inundative biological control, where rapid suppression of the host population is

most important, parasitoid species that host feed may have an advantage over species that do not because they can potentially cause deaths of more hosts than the number in which they would lay eggs alone.

8.2.1 Life history strategies in parasitoid communities

Many insect hosts have different parasitoids associated with different stages of their life cycles. Entomologists have been interested in the differences in life history strategies within parasitoid communities using the same host. Peter Price investigated a group of parasitoids attacking larvae of Swaine jack pine sawflies, *Neodiprion swaini* (with larval stages similar to caterpillars feeding externally on foliage) on pines in Quebec (e.g., Price, 1973) while Askew (1975) studied the parasitoids associated with oak galls and oak leaf miners in the United Kingdom (see Mayhew & Blackburn, 1999). There seemed to be a similar pattern in both systems, with “early succession” colonizers attacking larval hosts; these parasitoids had many eggs to lay (high fecundity) and their body sizes were larger but they were poor competitors. The smaller “late succession” colonizers used pupal hosts and had fewer eggs to lay but they were better competitors. From these relations, Price proposed a “balanced mortality hypothesis” stating that the fecundity level of the parasitoid species was balanced by the probability of survival of the progeny. Species using earlier host stages that would potentially be subject to mortality over a longer period of time had more eggs to lay. Species using later host stages, often in concealed locations that had less chance of being eaten or parasitized, laid fewer eggs. Later theorists connected the fact that most of the early succession colonizers were koinobionts and endoparasitoids while later succession colonizers are usually idiobionts and ectoparasitoids.

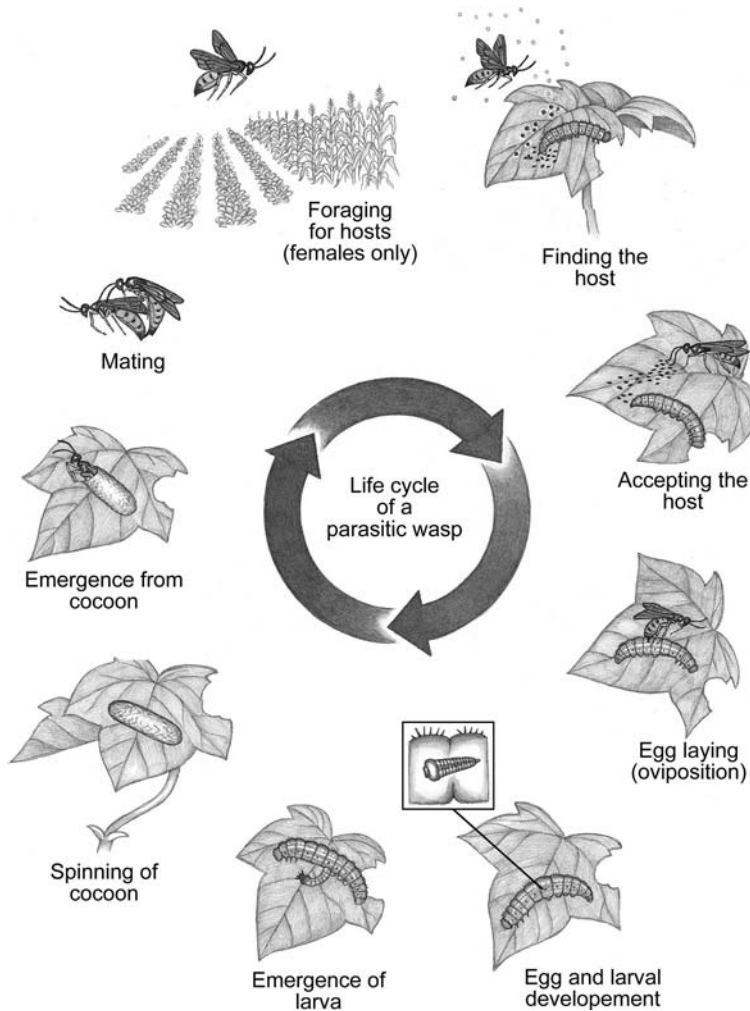
8.3 Locating and parasitizing a host

Successfully finding a host and parasitizing it are critical for reproduction by parasitoids. This can indeed be demanding for parasitoids with specialized requirements such as a limited number of acceptable host species, specific host life stages, or hosts with aggregated distributions. The process of locating and parasitizing hosts can be thought of in terms of a progression of several generalized steps: locating the correct habitat for the host, locating a host, and then evaluating the host to determine that it is the correct species, the right stage, and of sufficient quality for oviposition (Fig. 8.8).

8.3.1 Locating the host habitat over long distances

Even the largest parasitoids are relatively small and they face an environment that often contains few hard to find hosts with aggregated distributions. If parasitoids emerge where hosts of the correct stage

Fig. 8.8 Example of the life cycle of a parasitic wasp. The female finds a host she will identify by touching her antennae to the caterpillar's feces. She injects eggs into the caterpillar, then wasp larvae develop feeding within the caterpillar, pupate, and then emerge. From egg to adult death requires about 5 weeks. (Tumlinson et al., 1983.)



are present, habitat location is of course not a problem. However, if the host population at that site has declined or the correct host stage is not present, the parasitoids will need to find a new habitat occupied by hosts. Long-distance habitat location is difficult to study and not so well understood, but may involve a combination of visual, olfactory, and sometimes auditory cues. The most reliable cues for long-range orientation to locate the correct habitat would be cues originating from the host itself, although such cues might be difficult to detect over long distances. More likely, habitat location over long distances involves the more abundant cues associated with the host habitat, such as the food plant of a herbivorous host or yeasts associated with rotting fruit hosting fruit fly hosts.

8.3.2 Finding hosts

Once within the correct habitat, olfaction is widely used to find hosts. Parasitoids often use chemicals emitted by frass (larval faeces) or

honeydew, either as volatiles or on contact. Experiments with parasitic wasps have shown that both volatiles and contact chemicals can be involved in finding hosts. Researchers showed that wasps could learn that specific volatiles were associated with hosts (Tumlinson *et al.*, 1993). To investigate learning, researchers allowed parasitic wasps to touch non-volatile chemicals from frass from larval hosts with their antennae and simultaneously let them smell vanilla. From then on, wasps were attracted by the smell of vanilla because they had learned that this smell was associated with finding the correct host.

Pheromones (chemicals used by hosts to find mates or to aggregate) can also be used by parasitoids to find areas where hosts are present. In fact, host sex pheromones can be used by parasitoids that do not attack adult stages because susceptible eggs and early instars may be located in the same areas as adults. Locating plants being attacked by hosts has long been considered a major way that parasitoids find plant-feeding hosts. Recent research has shown that plants damaged by specific herbivore species emit distinct volatile profiles (sometimes distinct even among related species) and these specific volatiles may then attract specialist parasitoids. Tobacco or cotton plants infested with tobacco budworm (*Heliothis virescens*) or bollworm (*Helicoverpa zea*) produce different volatiles. The parasitoid *Cardiochiles nigriceps* is quite specialized, predominantly attacking tobacco budworm instead of bollworm. This parasitoid was attracted to plants on which tobacco budworm had fed much more than plants on which bollworm had fed. To demonstrate that the attraction was not due to the presence of the caterpillars, both caterpillars and damaged leaves were removed and the preference remained for plants on which *H. virescens* had fed, triggered only by the plant volatiles that had been induced by feeding (De Moraes *et al.*, 1998).

Smells of host body parts such as moth wing scales can also aid parasitoids in locating hosts. Chemicals extracted from wing scales from European corn borers are used by the egg parasitoid *Trichogramma nubilale* to remain in specific locations (Shu *et al.*, 1990). Some insects, such as pine looper moth (*Bupalus piniarius*) larvae, leave behind a trail when they move from place to place. This chemical trail originating from the caterpillar cuticle is followed by large solitary parasitoids (*Poecilostictus cothurnatus*) to locate hosts. Silk is used by many arthropods for concealing egg masses or pupae and is the source of volatile as well as contact chemicals used by some parasitoids to locate hosts.

In addition to chemical signals, host vibrations, visual cues, and acoustical signals are used. Host vibrations are often used by parasitoids attacking concealed hosts. Parasitoids of beetle larvae feeding beneath tree bark often stand motionless on the bark surface to sense vibrations caused by movements of larval hosts within the wood. There is some evidence that some parasitoids may find hosts by looking for irregular outlines of leaves, caused by feeding damage from hosts. Parasitoids can also use auditory cues to find hosts.

Tachinid flies (*Ormia ochracea*) parasitizing crickets (*Gryllus integer*) are attracted to male crickets singing to attract females. They lay living larvae on a singing male and the larvae quickly burrow inside to begin developing. Interestingly, satellite males that do not sing but try to steal females from singers are rewarded for their dastardly ways because they are rarely parasitized (Cade, 1975).

8.3.3 Accepting a host

Once potential hosts are located, they must be assessed by the parasitoid to determine whether this is the correct species and life stage. The host can be accepted when an adult female externally contacts the host or the female can wait until her ovipositor has been inserted into a potential host before deciding to lay an egg. Generally, for parasitic wasps attacking motionless hosts such as eggs, pupae, or scale insects, hosts are first examined by the parasitoid tapping or drumming the host with her antennae which are very sensitive to chemical cues telling the parasitoid if this host is the correct species and whether or not it has already been parasitized (Box 8.1). Alternatively, the ovipositor can be used to probe within the host before an egg is laid.

Although examination using the antennae is common, parasitoids attacking more active or aggressive hosts may take less time for evaluation and various of these acceptance steps may not occur. In the extreme case of parasitoids attacking active or predaceous hosts, specialized behaviors have often evolved so that parasitoids can successfully oviposit but also survive to lay more eggs. Parasitoids attacking ants are known to hover above potential hosts, much as a hawk hovers above its prey, and then quickly swoop down and oviposit very quickly before the ant can defend itself (Shaw, 1993).

8.4 | The battle between parasitoid and host

Factors determining which host species can be attacked by a parasitoid can be varied and often poorly understood. Host range for any parasitoid species is likely to be the outcome of the interplay between adaptations of the parasitoid to subdue, attack, and develop in particular hosts and adaptations by hosts to repel or resist the parasitoid. The diversity of reciprocal offense and defense that have been developed in this coevolutionary arms race is truly amazing.

8.4.1 Host defense

Many different specialized features of insect species probably act as deterrents to parasitoids. Thick and hard host egg shells and cuticles or long hairs can act as physical barriers. Mobile hosts defend themselves by thrashing, kicking, shaking, dropping on silk threads, or simply falling when parasitoids attack. Hosts living in a concealed location such as a rolled leaf or leaf mine certainly gain some protection from these specialized habitats but some parasitoids, in turn,

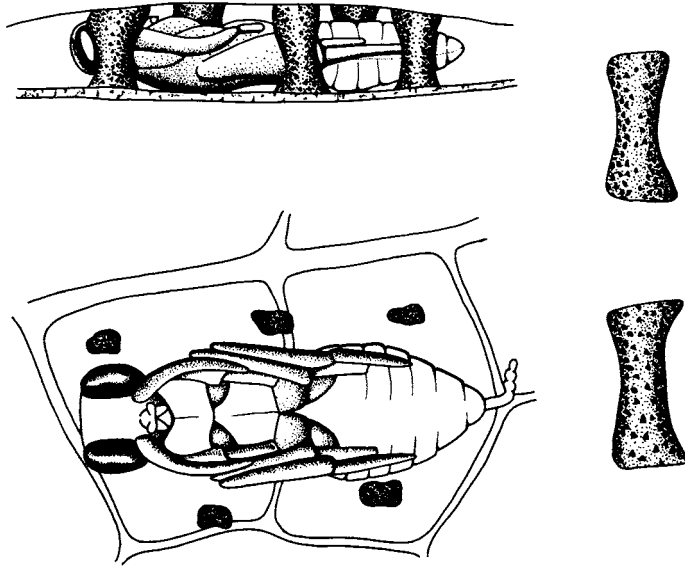


Fig. 8.9 Supporting props of fecal matter made by the parasitoid *Chrysocharis gemma* for pupation, so that as a leaf dries out, the pupation chamber within the leaf does not collapse. (Viggiani, 1964.)

have evolved methods for overcoming these defenses. One small wasp parasitoid of leaf miners has solved the problem of how safely to pupate within a flat leaf mine constructed between the two surfaces of a leaf. The larvae utilize their faecal material, which hardens as props to create a safe space within the leaf where the defenseless pupa will not be squashed while metamorphosis occurs (Fig. 8.9).

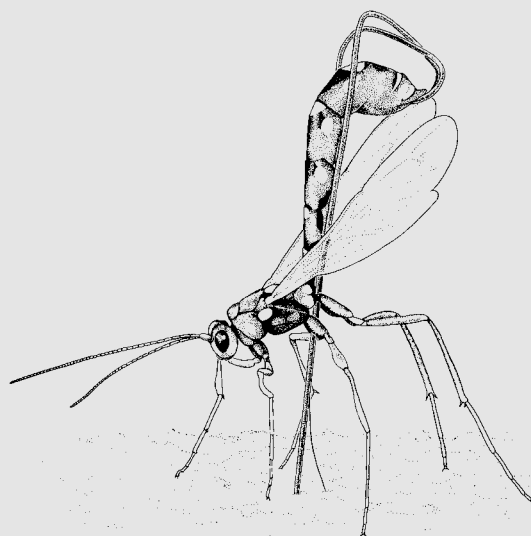
Hosts can be defended by other members of the community, sometimes quite effectively. Ants that tend aphids for the honeydew they produce may also protect the aphids from natural enemies. Some hosts feed on plants with secondary plant compounds that are known to be toxic to many insects and they sequester these compounds. These so-called “nasty hosts” can then influence the developmental success of parasitoids; several species of parasitoids develop poorly within caterpillars with high levels of nicotine from feeding on tobacco plants (Barbosa *et al.*, 1986, 1991). Although caterpillars can use these plant defenses to protect themselves, some parasitoids have evolved the ability to tolerate such compounds, thus utilizing a host with fewer competing parasitoids.

8.4.2 Parasitoid attack

Parasitoids have developed specialized methods for successfully ovipositing in and on acceptable hosts. Ovipositors differ in length and morphology, so that parasitoids are able to lay eggs in or on their hosts, whether exposed or concealed deep within a habitat. Oviposition by some species can be very slow, requiring up to 30 minutes for the delicate ovipositor to drill with precision deep within tree trunks to lay eggs on host larvae feeding within (Box 8.3). At the other extreme, phorid flies in the genus *Pseudacteon* can lay an egg in the thorax of a fire ant (*Solenopsis* spp.) in less than 1 second (Morrison *et al.*, 1997), presumably thus avoiding mass attack by the ant colony.

Box 8.3 Parasitoids within trees: how do they get there?

Some specialized challenges have been overcome by parasitoids attacking hosts that develop deep within tree trunks. These parasitoids are so difficult to study that complete information is not available regarding the biology of any one species. In early June in forests in northeastern North America, you might be lucky enough to see *Megarhyssa atrata*. This group of parasitoids within the Ichneumonidae are among the largest parasitoids (4–5 cm long with an ovipositor the same length). This group of parasitoids attacks larvae of wood wasps and horntails (Xiphydriidae and Siricidae) that develop deep within rotting trees. Males of *Megarhyssa* emerge before females in spring and groups of males can be found aggregated around certain locations on the trunks of dying trees. Look closer and you'll find a small hole chewed in the tree trunk. A few lucky males will have extended their abdomens into that hole where, further inside the tree within a tight gallery, a female will be mating with one of these males. The female will eventually continue to emerge through the hole and, once she is out, will mate again. Then, she will fly away to find the correct locations to lay eggs.



Female of the parasitic wasp *Megarhyssa nortoni* probing a pine log with her ovipositor in search of a larva of the wood wasp, *Sirex noctilio*. (Illustration by Karina H. McInnes, Gullan & Cranston, 2000.)

But how will the female find these specialized hosts in such hidden locations? The immatures of the pine wood-wasp (*Urocerus gigas*) live deep within tree trunks in association with a symbiotic fungus (*Amylostereum chailloti*) injected by their mothers that rots the wood. This fungus provides an olfactory cue to help adult female parasitoids find the cryptic locations of immature wood wasps. A female *Rhyssa persuasoria* uses the tips of her long antennae to find an exact location where a host larva of *U. gigas* is located within the tree trunk and she begins drilling with her ovipositor. She orients her body so that her ovipositor is perpendicular to the tree trunk, supported by the ovipositor sheaths and the

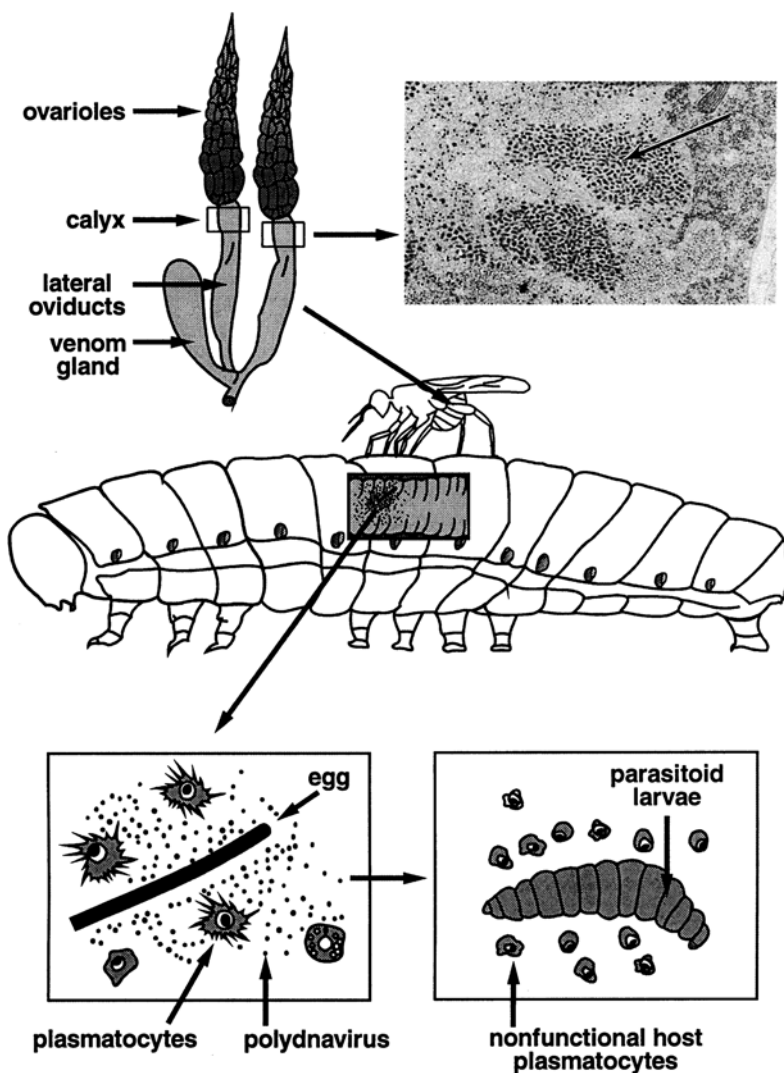
bases of her hind legs. The tips of her ovipositor have cutting ridges and she cuts through the wood by working the two parts of the ovipositor back and forth, sawing into the wood. This is difficult and slow work that can take >5 hours; for adult female *Rhyssa persuasoria*, the full length of the ovipositor (4–5 cm) usually must be inserted to reach a potential host. When a host is located, the female stings it to paralyze it before laying an egg. The egg passes down the egg canal in the middle of the ovipositor and is laid on top of the host where it will hatch and develop as an ectoparasitoid.

Ectoparasitoids attacking concealed hosts often inject a permanent toxin that paralyzes the host to prevent host movement and molting. This makes sense because movements by the hosts in small spaces could damage externally attached parasitoid larvae that are quite incapable of defending themselves.

In contrast, endoparasitoids must contend with living within a host. Insect immune systems are less complex than mammalian immune systems but insects can still effectively mount an assault on a parasitoid egg or immature parasitoid larva if their host blood cells recognize the intruder as non-self. If a parasitoid egg or larva is recognized as non-self, insect blood cells (hemocytes) can spread over the surface of the invader, effectively walling it off. The subsequent capsule of blood cells surrounding the invader often turns black in a process called melanization and the parasitoid within is killed, probably either by asphyxiation or due to the toxic quinones produced during the blackening process (Strand & Pech, 1995). Of course, endoparasitoids have developed methods for avoiding capsule formation, or encapsulation. There seems to be variability among closely related parasitoids in whether they are encapsulated or not when within hosts. It has been hypothesized that some parasitoids escaping encapsulation have surfaces that are not recognized by the host as being foreign; in essence, the parasitoid can grow, undetected, within the host. Alternatively, some tricky species deposit their eggs within host tissues, such as the nervous tissue, the gut wall, and the fat body, so the circulating blood cells that would be able to recognize them as non-self do not contact them.

To prevent encapsulation, members of the larger ichneumonoid wasps have teamed up with viruses, named polydnnaviruses, to help them survive within hosts. Adult females inject a polydnnavirus into the host when ovipositing and the virus, along with additional materials injected by the female, acts to generally block the host immune response, including preventing melanization and reducing the number of responsive blood cells (Fig. 8.10). The virus is just along for the ride and does not reproduce in the parasitized insect. The virus is incorporated in the parasitoid DNA and only reproduces within the reproductive tract of the adult female wasp. This strategy ensures distribution of this specialized virus to wasp progeny thus also ensuring survival of the host-dependent virus.

Fig. 8.10 Polydnavirus–parasitoid–host relations. Polydnavirus is produced in the calyx region of the ovaries of an adult female parasitoid and is released within the host during oviposition to subsequently suppress the immune cells (plasmatocytes) of the parasitoid host. (Courtesy of Boucias & Pendland, 1998.)



8.5 | Use of parasitoids in biological control

Parasitoids have been central to the growth of biological control of arthropods. In particular, parasitoids that are prolific and actively search for and find hosts have been prized. Parasitoids that have been used for biological control are frequently those that are more host specific, so that their offspring all target the pest.

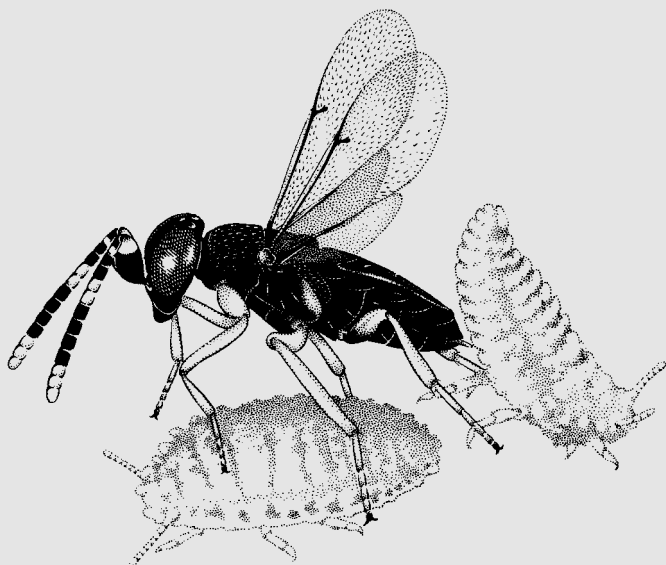
8.5.1 Classical biological control

Parasitoids have been used extensively for classical biological control. The high degree of host specificity characteristic of many parasitoid species makes these natural enemies first choices for classical biological control introductions. In a recent estimation, a total of 907 species of parasitoids have been introduced for classical biological

control. The majority of these, 765 species, were parasitic wasps and 125 were parasitic flies. Parasitoids have been used for good reason because there are many success stories from programs using parasitoids. Among the many successful classical biological control programs, several are described in Caltagirone (1981) and DeBach & Rosen (1991), and a fairly recent example of an introduction of a tiny parasitic wasp for control of cassava mealybug in Africa is described here (Box 8.4).

Box 8.4 Controlling the introduced cassava mealybug in Africa

Around the same time that the cassava green mite (Box 7.3) was first found in Uganda, cassava mealybug, *Phenacoccus manihoti*, was first found in the Congo. As with cassava green mite, this pest rapidly spread through central Africa and, by 1986, the cassava mealybug occurred in 25 African countries, causing cassava yields to decline drastically.



A female *Apoanagyrus lopezi* examines potential cassava mealybug hosts with her antennae and is shown inserting her ovipositor to lay an egg. (van Alphen & Jervis, 1996.)

A classical biological control program was begun and the first step was to search for natural enemies (Neuenschwander & Herren, 1988). There are many species of *Phenacoccus* in central and northern South America, the hypothesized home of *P. manihoti*. Scientists searched for *P. manihoti* to collect its natural enemies in Central America, northern Colombia and Venezuela. They found a mealybug initially thought to be the new African pest. Several parasitoid species from this mealybug were collected but they would not reproduce in *P. manihoti* in the insectary in the Congo. On closer examination, researchers found that there were both males and females of the South American mealybug that had been collected, while the African

pest had only females, and these two mealybugs also differed in morphology. It was decided that the mealybug that had been collected was not the African pest. Foreign exploration continued in South America and *P. manihoti* was finally found, only in limited areas of Paraguay, Bolivia and southern Brazil. Where it occurred, *P. manihoti* populations were hard to find. A highly host-specific parasitic wasp, *Apoanagyrus lopezi*, was found and first released in 1981. This parasitoid proved exceptionally effective at becoming established and controlling cassava mealybug. Attraction of these small wasps to the wax produced by cassava mealybugs helps them find hosts. This species parasitizes cassava mealybugs but also host feeds, which adds to mortality of hosts. Mass rearing methods were developed once it became evident that this wasp was particularly effective but would not spread so quickly on its own. However, distributing this wasp throughout the area infested with the pest was a problem; while the wasps spread on their own through agricultural areas, spread through rainforest zones was slower. To facilitate spread, wasps were mass-produced in insectaries, transported by air, and then released on the ground. In some more remote areas, methods were developed to release wasps from aircraft flying over cassava-growing areas. Between 1981 and 1990, *A. lopezi* was released in over 100 areas and was documented as becoming established in 24 African countries.

With the great diversity in life history strategies among parasitoids, it has often been difficult to make decisions regarding which species to introduce. Recent models have demonstrated that making such a decision is, in fact, quite difficult and optimally requires information about such factors as fecundity, host feeding, and response to host density. Surprisingly, results from models disproved the dogma that host feeding parasitoids are always superior; host feeders frequently have lower fecundity and they require higher host densities before depressing pest populations.

8.5.2 Augmentative releases

The other principle use of parasitoids has been their extensive development for augmentative releases, either inoculative or inundative. All parasitoids that have been developed for these purposes are parasitic wasps and predominantly include species with idiobiont strategies; since these species develop within hosts that are inactive, producers do not have to worry about feeding the hosts after they are parasitized and this facilitates mass-production.

Augmentative use requires mass production of parasitoids, first producing hosts and then exposing them to parasitoids. In most cases, after parasitized hosts die but while parasitoids are still inside the hosts, they are released for control. In field crops, tiny parasitoids attacking eggs of caterpillar pests, *Trichogramma* species, are being developed for control and have been used extensively in some systems. A survey published in 1994 cited the greatest use in the former USSR, followed by China and Mexico (Li, 1994). *Trichogramma* species have been used extensively in corn, rice, sugar cane, cotton, vegetables, and pine forests and the pests most often targeted have been corn borer, sugarcane borer (*Diatraea saccharalis*), and cotton bollworm. For

Table 8.2 Common parasitic wasps used for augmentative releases

Group	Species	Host	Use area ¹
<i>Ichneumonoidea</i> Aphidiidae	<i>Aphidius colemani</i>	Aphids	I
	<i>Aphidius matricariae</i>	Aphids	I
<i>Chalcidoidea</i> Aphelinidae	<i>Aphytis melinus</i>	Scale insects	O
	<i>Encarsia formosa</i>	Whiteflies	I
Pteromalidae	<i>Eretmocerus eremicus</i>	Whiteflies	I
	<i>Muscidifurax raptor</i>	House flies	I
	<i>Muscidifurax raptorellus</i>	House flies	I
	<i>Muscidifurax zaraptor</i>	House flies	I
	<i>Nasonia vitripennis</i>	House flies	I
	<i>Spalangia cameroni</i>	House flies	I
	<i>Trichogramma brassicae</i>	Moth eggs	O
Trichogrammatidae	<i>Trichogramma minutum</i>	Moth eggs	O
	<i>Trichogramma ostriniae</i>	Moth eggs	O
	<i>Trichogramma pretiosum</i>	Moth eggs	O

¹ I = Indoors, referring to greenhouses, interior landscapes or facilities for raising poultry or livestock;
O = Outdoors, referring to crops and gardens.

From C. Glenister, IPM Laboratories, pers. commun.

all of these species, the larval stages that cause damage are hidden within the plant and thus are very difficult to control using chemical pesticides. Using an egg parasitoid is wise because pests are then killed before they develop to a stage causing damage.

The chalcidoid *Encarsia formosa* is widely used by the nursery industry to combat the ubiquitous greenhouse whitefly (Box 8.5). As of 2000, a leading producer and distributor of biological control agents in Europe marketed 11 different species of parasitoids, among a total of 36 species of natural enemies available predominantly for insect and mite control in greenhouses. Some parasitoid species commonly used in commercial biological control in the United States are listed in Table 8.2. As is evident, only parasitic Hymenoptera and not Diptera or other taxonomic groups with a parasitoid life strategy are produced for inoculative and inundative releases.

Box 8.5 *Encarsia formosa* against greenhouse whitefly

The greenhouse whitefly (*Trialeurodes vaporariorum*) has a huge range of plants on which it feeds but, in greenhouses, it is predominantly a major pest of vegetable crops. This pest sucks the phloem sap of plants and excretes sugar-laden honeydew that falls on the foliage below. The major injury from this pest is caused by sooty mold growing on the honeydew and subsequently reducing photosynthesis and respiration of the plants (Parrella *et al.*, 1999).

In greenhouses, there can be numerous pests on the same crop and spider mites are fairly regular culprits. Spider mites developed resistance to chemical insecticides in Europe beginning in 1949 so growers began using predatory mites for control. After greenhouse whiteflies emerged as a pest, growers had to use natural enemies to control greenhouse whitefly or spider mite control by predaceous mites was disrupted. The small parasitoid *Encarsia formosa* had first been investigated for control as early as 1927. This parasitoid can be very effective at controlling greenhouse whitefly. It uses the presence of immature whiteflies or honeydew to locate hosts. Adult females do not lay eggs in hosts that have previously been parasitized, thereby avoiding wasting eggs. As an added benefit, adult females can



The whitefly parasitoid *Encarsia formosa*. (Photo taken by P. Sutherland; van Lenteren & Martin, 1999.)

host feed on unparasitized hosts and thus kill hosts that are not used for progeny production.

It was only in the 1970s that efficient and predictable control resulted after development of precise recommendations for use of *E. formosa* in the United Kingdom and Netherlands. Use of this parasitoid increased so that in 1985 alone it was estimated that 1600 ha of greenhouses were treated with *E. formosa*, and this parasitoid continues to be widely used for whitefly control in greenhouses. *E. formosa* is often inoculatively released, and several specific methods for application have been suggested. For one, successive regular introductions of *E. formosa* begin soon after the crop has been planted or when the pest is first observed (blind releases). As an alternative, plants with established populations of greenhouse whitefly parasitized by *E. formosa* are placed in the greenhouse throughout the year as banker plants.

FURTHER READING

- Askew, R. R. *Parasitic Insects*. New York: American Elsevier, 1971.
 Clausen, C. P. *Entomophagous Insects*. New York: Hafner, 1972.
 Godfray, H. C. J. *Parasitoids: Behavioral and Evolutionary Ecology*. Princeton, NJ: Princeton University Press, 1994.

- Hassell, M. P. & Godfray, H. C. J. The population biology of parasitoids. In *Natural Enemies: The Population Biology of Predators, Parasites, and Diseases*, ed. M. J. Crawley, pp. 265–292. Oxford: Blackwell Scientific Publishers, 1992.
- Hawkins, B. A. *Pattern and Process in Host-Parasitoid Interactions*. Cambridge: Cambridge University Press, 1994.
- Hochberg, M. E. & Ives, A. R. (eds). *Parasitoid Population Biology*. Princeton, NJ: Princeton University Press, 2000.
- Quicke, D. L. J. *Parasitic Wasps*. London: Chapman & Hall, 1997.
- Waage, J. & Greathead, D. (eds). *Insect Parasitoids*. New York: Academic Press, 1986.
- Wajnberg, E. & Hassan, S.A. (eds). *Biological Control with Egg Parasitoids*. Wallingford, UK: CAB International, 1992.

Chapter 9

Parasitic nematodes

The Phylum Nematoda is exceptionally diverse, including species adapted to just about every type of life style imaginable. It is no surprise that many of these roundworms have adapted to lives as parasites of invertebrates. Nematodes, or roundworms, that attack arthropod pests range in size from those visible without magnification to microscopic species. They have reduced morphological features but one feature common to these species is that all are long and thin. Many can only enter hosts through body openings (mouth, anus, spiracles) or wounds, after which they often penetrate the body cavity (hemocoel). Others enter the host gut passively when nematode eggs are ingested with food. Some have a hardened stylet or spear in their mouths that they use to penetrate actively through arthropod cuticle.

Nematodes hatch from eggs and molt from one to another of four juvenile stages before molting to adults. For some species, adults are either male or female (amphimictic) while in others, adults are hermaphroditic, with each individual having reproductive organs of both sexes. The nematode life cycle is often ordered such that only a specific stage, often a juvenile called an infective juvenile, will leave a host to find a new host to infect. However, such departures only happen when nutrients within a cadaver are exhausted.

All nematodes are basically aquatic, requiring at least a film of water in which to live, although some insect parasitic nematodes can tolerate moderate desiccation. Of course, while nematodes are living within arthropods or cadavers of arthropods their surroundings are moist. During dispersal, nematodes are more at risk and for this reason many species occur in aquatic habitats or in the soil. Nematodes display some ability for finding or attacking hosts. Some colonize the ovaries of adult female hosts after infection and take advantage of the oviposition behavior of hosts so that when hosts lay eggs, infective juveniles are deposited instead (“nemaposited”) in locations where healthy hosts would occur. Other nematodes actively search for hosts, for example, some species of *Steinernema* and *Heterorhabditis* are attracted by host fecal components, bacterial gradients, plant roots, or carbon dioxide.



Fig. 9.1 Infective juvenile nematode of *Steinernema carpocapsae*. (Photo by Patricia Timper; Hoffmann & Frodsham, 1993.)

Life history strategies of nematodes attacking invertebrates are diverse, ranging from species that live within hosts as parasites but do not cause mortality to species whose symbiotic bacteria kill hosts quickly.

9.1 | Steinernematidae and Heterorhabditidae

The families Steinernematidae and Heterorhabditidae have been quite intensively studied due to their importance in biological control of insects. Individuals of these nematode species are very small, less than 1–3 mm long (Fig. 9.1). These two families, both within the Order Rhabditida, are not especially closely related, yet they have adopted very similar life history strategies. For *Steinernema*, both a male and a female nematode must enter a potential host for reproduction to take place, while for *Heterorhabditis*, all infective juveniles become hermaphrodites so only one individual is required to infect a new host for reproduction to ensue. Juveniles can remain within the mother, basically parasitizing her, only leaving once they themselves become adults.

A unique aspect of the biology of these nematodes is their symbiosis with bacteria. The third-stage infective juveniles carry symbiotic bacteria in their guts and, after invading a host, release the bacteria. These bacteria, species of *Xenorhabdus* for Steinernematidae and *Photorhabdus* for Heterorhabditidae, are responsible for killing hosts very rapidly, within 2–3 days. Mortality of host insects is caused by a toxin that kills the host. The bacteria then proceed to increase, using the cadaver for nutrients, and the nematodes principally feed on the bacteria. Nematode generations continue to develop within the same cadaver until nematode density is high and nutrients are low, at which time infective juveniles exit to find a new host, taking some of the bacteria along with them in their guts (Fig. 9.2). Until the infective juveniles are ready to leave, cadavers of insects killed by these nematode–bacterial associations remain intact, although they are flaccid (Fig. 9.3). Cadavers of insects killed by *Heterorhabditis*/*Photorhabdus* can be identified because they often

Fig. 9.2 Life cycle of a steinernematid or heterorhabditid nematode. (Drawing by A. E. Burke.)

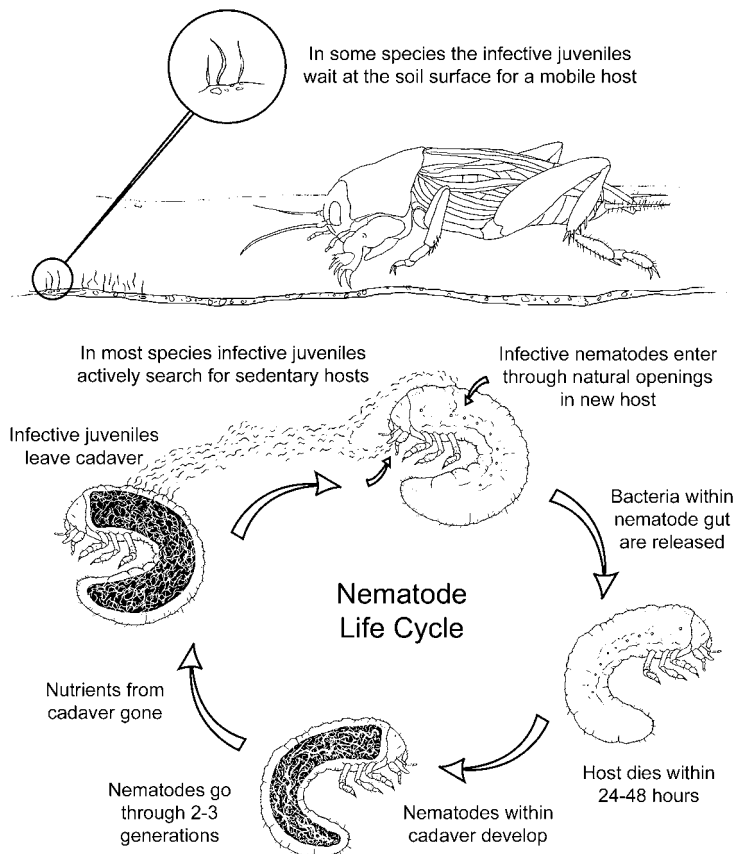
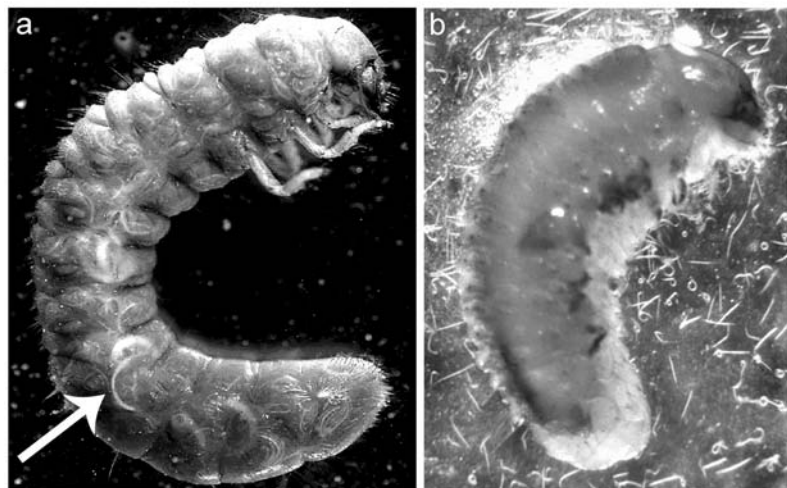


Fig. 9.3 a. Dead Japanese beetle grub (*Popillia japonica*) filled with individuals of the entomopathogenic nematode *Heterorhabditis bacteriophora*. Larger, white adult nematodes can be seen through the cadaver cuticle. (Photo by J. Ogrodnick in Vittum et al., 1999). b. Infective juveniles of *Steinernema riobrave* emerging from dead citrus root weevil (*Diaprepes abbreviatus*) larva. (Photo courtesy of C. McCoy; McCoy et al., 2000.)



turn orange to red, due to pigments produced by the bacteria, and cadavers can luminesce for a short time. Also, the interior of the cadaver is quite gummy, in comparison with cadavers of insects killed by *Steinernema/Xenorhabdus*.

The association between the nematodes and bacteria is mutualistic because both members benefit. Although the nematodes can kill the host in the absence of bacteria, they do so slowly. They cannot reproduce without feeding on the bacteria that supply them with required nutrients, such as sterols. With these bacteria, hosts are killed much more quickly and cadavers are kept free of other bacteria due to antibiotics produced by the symbiotic bacteria. The bacteria gain from the association because they cannot disperse, locate a new host insect, and invade the hemocoel on their own so the nematodes provide transport.

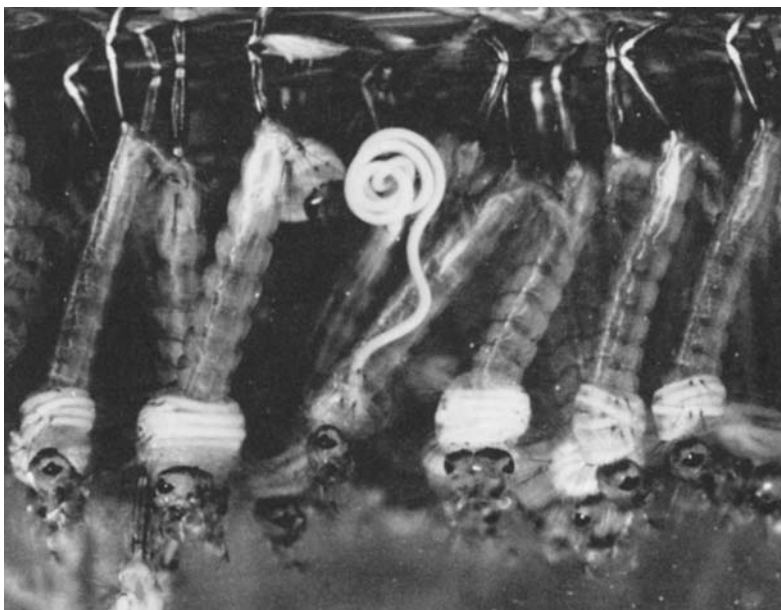
Steinernema and *Heterorhabditis* species can have very different strategies for locating hosts. Most species of both genera actively search for hosts and target sedentary hosts in the soil and these nematodes have been named cruisers. To locate and infect mobile hosts, some species of *Steinernema* display a radically different behavior. These ambushers go to the surface of the soil and stand vertically on their posterior ends on the top of soil particles, waving back and forth, or just standing still (Fig. 9.2). If a host walks over the ambusher, the nematode attaches to it, even jumping if the host is nearby but not in contact.

Steinernematids and heterorhabditids survive for only a few hours on exposed surfaces. They are basically soil-dwelling and can be greatly affected by exposure. As basics, they require moisture and oxygen. Dry soil can seriously impair mobility and survival, but nematodes within dry soil can often persist for 2–3 weeks. If nematodes are dried slowly they adapt and can enter a quiescent or dormant state that is more desiccation-tolerant. Soil structure can also greatly influence these nematodes, with enhanced movement and survival in lighter soils where there are larger pore spaces.

Only 35 species have been described in the families Steinernematidae and Heterorhabditidae (Adams & Nguyen, 2002). All are parasites of insects but they vary in their degrees of host specificity. Some have rather large host ranges while others seem to be more specific. Host specificity can be influenced at several levels. Some insects groom to remove nematodes before they penetrate and many soil-dwelling larvae have fine sieve plates covering their respiratory openings that restrict nematode entry by that route. Some insects have very thick and convoluted gut walls that are thought to deter penetration.

It is common for *Steinernema* and *Heterorhabditis* to arouse a defense response by insect blood cells once within the body cavity of a potential host. Some nematodes can become encapsulated, just as with parasitoid larvae, but other nematode species are not encapsulated, probably because they are not recognized as foreign by the blood cells. Sometimes *Steinernema* or *Heterorhabditis* can overwhelm

Fig. 9.4 Juvenile mermithids (*Romanomermis culicivorax*) coiled within the thoraces of mosquito larvae (*Culex pipiens quinquefasciatus*) during laboratory mass rearing. One postparasite is just emerging. (Photo by J. J. Peterson in Poinar, 1979.)



the encapsulation response; if enough nematodes enter the insect, there are not sufficient blood cells to encapsulate all of them at the same time. As a third alternative, the nematode or the bacteria may avoid encapsulation by suppressing the immune response of a potential host.

9.2 | Mermithidae

A diversity of other groups of nematodes lives more as long-term parasites within hosts and some are even ectoparasitic, attached to the surfaces of hosts. Among these, the best known are the Mermithidae, obligate parasites that live in a variety of invertebrate hosts. Mermithid adults are macroscopic, with adult females often 5–20 cm or more in length, although still very thin. They have been of great interest for biological control because hosts usually die once mermithids complete their development, leave the host, and enter the environment.

We know quite a bit about mermithid species attacking mosquitoes, black flies, leafhoppers, and grasshoppers. For the mosquito-pathogenic *Romanomermis culicivorax*, juveniles live within mosquito larvae for only a few weeks (Fig. 9.4), after which they emerge as postparasites, killing hosts as they emerge. They drop to the sediment at the bottom of aquatic habitats, develop into males and females, then mate and lay eggs that overwinter to produce infective juveniles the next spring.

9.3 | Use for control

Development of nematodes for biological control is an active area, concentrating on use of nematodes for control of slugs and soil-dwelling insects. In addition, nematodes have been used for control of pests in cryptic habitats, where the nematodes are protected from environmental extremes.

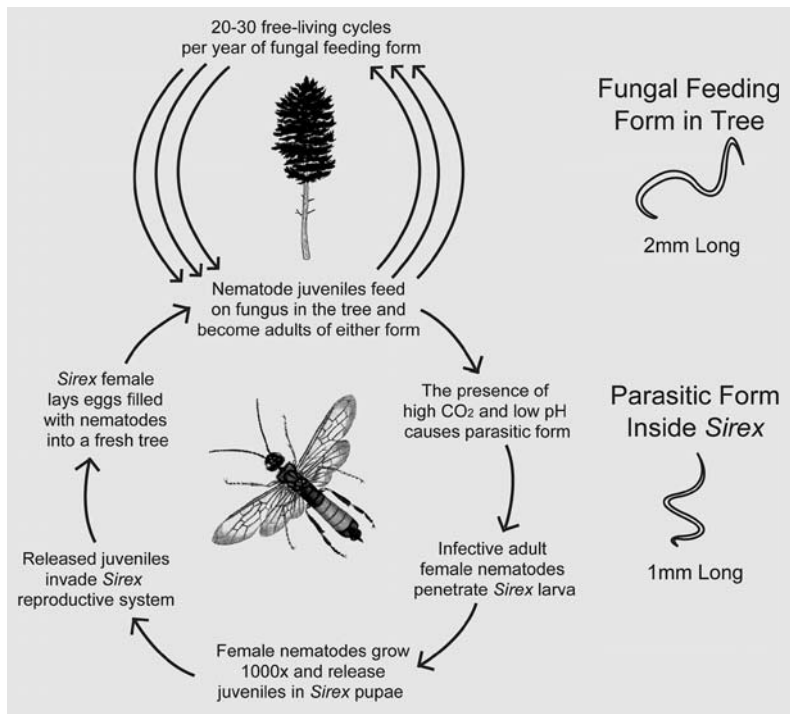
Nematodes have been used for classical biological control in only a few instances, but some programs have been extremely successful. *Beddingia siricidicola* (Family Phaenopsitylenchidae) has been released throughout much of Australia against the introduced wood wasp *Sirex noctilio* that bores within pine trees (Box 9.1). Another successful program involved control of mole crickets introduced from South America to the lawns and pastures of Florida. *Steinernema scapterisci* from Uruguay and Argentina was introduced, became established and spread, resulting in up to 98% decreases in mole crickets over 3 years in some locations. However, in scattered lawn environments treated in various ways for multitudes of problems, *S. scapterisci* has not always persisted. For a more short-term approach, when this nematode is applied inundatively (2.5×10^9 nematodes/ha) it can provide the same level of control as standard insecticides.

Box 9.1 | *Beddingia siricidicola* and *Sirex noctilio*

The native European *Sirex noctilio* can kill healthy pine trees. A female *S. noctilio* drills with its ovipositor 2 cm deep in the wood of a healthy tree and injects spores of a symbiotic fungus (*Amylostereum areolatum*) at the same time as laying its egg. The fungus is a tree pathogen and starts growing, eventually spreading throughout the tree and killing it. The wood wasp egg is stimulated to hatch by the presence of the fungus and the larva develops by boring through the tree in association with the fungus. This pest was introduced to Australia in the early 1960s and, as it spread, it left a wake of dead Monterey (= *radiata*) pines. Unfortunately, the Australian forestry industry had planted huge areas of Monterey pine, so there were plenty of hosts.

In Europe, researchers found that the nematode *Beddingia* (previously *Deladenus*) *siricidicola* killed these wood wasps, so it was decided to introduce this nematode to Australia. To start its life cycle, dispersal stage juvenile nematodes produced within an adult female wood wasp travel within her and invade her ovaries, with the result that each wood wasp egg can contain up to 200 juvenile nematodes. Parasitized adult female wood wasps still have the urge to oviposit, so they travel to healthy trees where they deposit nematode-filled eggs. Unparasitized wood wasps also lay eggs in these same trees and their healthy offspring become parasitized by nematodes. An adult nematode enters a healthy wood wasp larva by directly drilling through the body wall and then grows and reproduces within, without killing the larva.

It is difficult to imagine that the relatively few nematodes deposited when a female wood wasp nematoposits would be able to find the healthy larvae living within



Life cycle of the entomopathogenic nematode *Beddingia siricidicola*, which can live as a parasite of the European wood wasp *Sirex noctilio* or can feed on fungi within pine trees. (Adapted from Bedding, 1993.)

a tree. However, this nematode species can also develop solely by feeding on the wood wasp's symbiotic fungus. If the nematodes that emerge from eggs drilled into the wood are not stimulated by low pH and high carbon dioxide, they develop as fungus-feeders. The fungal-feeding nematodes then reproduce and increase within the tree until juveniles receive the proper stimuli to become parasitic.

The fact that this nematode has two options for its life cycle, parasitizing *S. noctilio* larvae and feeding on the symbiotic fungus, has been lucky for biological control efforts. The symbiotic fungus is fairly easy to grow in the laboratory and the nematodes are easily grown on the fungus. Initially, nematodes were mass-produced on lab-produced fungus but methods were improved with development of a more-efficient and less-expensive medium.

At the outset of the project to release *B. siricidicola* in Australia, large efforts were directed toward choosing the best nematode species and strain within species. The nematode strain that was chosen for mass release causes total sterilization of adult female *S. noctilio*, does not interfere with flight ability, and is compatible with parasitoids released against this host. *B. siricidicola* was extremely effective but, because it was thought that long-distance spread of this nematode would be slow, the nematode was produced in the laboratory and distributed through large biological control programs. While techniques for distribution were perfected during this time, the efficacy of the nematode slowly declined. By the time this was noticed, after 20 years of mass production and distribution, nematode efficacy had

dropped from 100% parasitism to 25% parasitism. Research demonstrated that the nematodes had become very efficient at growing while feeding on media but were less virulent as parasites. This problem was rapidly corrected by reisolating the nematode from the original release site and changing production methods. Today, host virulence is maintained because ample insect-virulent nematode cultures were frozen and each year a new virulent culture is thawed to use for mass production. Thus, before the virulence of that strain of nematode declines, a newly thawed, virulent culture is substituted for the culture that had been used over the past year.

Emphasis with *Steinernema* and *Heterorhabditis* has purely been on their mass application as biopesticides. This has been possible because they are easily grown in large quantities on inexpensive media. During initial investigations, these species were grown using solid media of pork kidney or dog food but mass-production technology subsequently became more sophisticated. Mass-production by larger industries has involved production in liquid medium in small vessels or fermentation tanks with capacities of up to 15,000 liters or more, yielding *c.* 10^5 juveniles per milliliter (Friedman, 1990). Cottage industries producing nematodes still use solid media or insect hosts. Nematodes may be formulated by absorbing a highly concentrated nematode suspension onto a porous material (often something like a sponge or foam) to provide ample oxygen so that nematodes survive well (Smart, 1995). Some nematodes are partially desiccated and mixed in powders or granules based on vermiculite or similar carriers. Methods for optimizing formulation, packaging and shelf life were critical for development of these nematodes for control because they must be living when applied. These nematodes are generally more effective if applied to looser, moist soils at moderate to warm soil temperatures. During application, using enough water to wash the nematodes off the soil surface into the soil is critical.

Work on use of Steinernematidae and Heterorhabditidae for control has only proceeded in earnest since the 1960s and 1970s. Nine species have been commercialized to various degrees, with much of this commercial expansion in the 1990s (Table 9.1). Research has shown that species vary in efficacy against different pests, both due to strategy for host location and inherent adaptation to host species (Box 9.2). Earlier attempts to use entomopathogenic nematodes against foliage-feeding insects were not successful. Pests against which nematodes are used include caterpillars, beetle grubs, flea larvae, and fly maggots associated with soil or in cryptic and moist habitats, for example insects boring in stems. Use of nematodes has predominantly targeted more specialized applications, such as pests in greenhouses, nurseries, turf, and cranberry bogs. Nematodes are simple to apply because they are compatible with conventional spray equipment and many pesticides. After application, in many instances the nematodes become established and will recycle in the pest population. Some species more widely used for control have broad host ranges and their

Table 9.1 Commercialized species of nematodes and their target hosts¹

Pest	System	Nematode
<i>Insecta</i>		
<i>Orthoptera</i> (Grasshoppers and crickets)		
Mole crickets	Turf	<i>Steinernema riobrave</i> , <i>S. scapterisci</i>
<i>Lepidoptera</i> (Caterpillars of moths)		
Armyworms, cutworms, webworms	Turf	<i>S. carpocapsae</i>
Artichoke plume moth	Artichoke	<i>S. carpocapsae</i>
<i>Coleoptera</i> (Beetles)		
Billbugs (Weevils)	Turf	<i>Heterorhabditis bacteriophora</i> , <i>S. carpocapsae</i>
Cranberry girdler	Cranberries	<i>S. carpocapsae</i>
Root weevils	Berries	<i>H. bacteriophora</i>
Root weevils	Citrus	<i>S. riobrave</i> , <i>H. indica</i>
Root weevils	Cranberries	<i>H. bacteriophora</i> , <i>S. carpocapsae</i>
Root weevils	Ornamentals	<i>H. bacteriophora</i> , <i>H. megidis</i>
Scarabs	Turf	<i>H. bacteriophora</i> , <i>H. megidis</i> , <i>S. glaseri</i>
Wood borers	Ornamentals	<i>S. carpocapsae</i> , <i>H. bacteriophora</i>
<i>Diptera</i> (Flies)		
Fungus gnats	Mushrooms, ornamentals	<i>S. feltiae</i>
<i>Gastropoda</i>		
Slugs and snails	Variety of field crops	<i>Phasmarhabditis hermaphrodita</i>

¹ Not all nematodes are available in all countries. Commercialized nematodes belong to the genera *Steinernema*, *Heterorhabditis* or *Phasmarhabditis*.
Grewal, 2002; A. Koppenhofer, pers. commun.

use must therefore be integrated with that of other soil-active natural enemies.

Box 9.2 | *Steinernema riobrave* fights citrus root weevil

A root weevil, *Diaprepes abbreviatus*, was first found attacking Florida citrus in 1964. Economic damage is caused by larval feeding and problems can be exacerbated if the citrus tree root pathogens (*Phytophthora* spp.) enter the wounds in the roots. Soil-dwelling pests are notoriously difficult to control and using a nematode that could search for weevil larvae seemed like a good idea. Several different species of *Steinernema* were investigated until a front-runner was discovered in the newly identified *Steinernema riobrave*, which is highly virulent against this pest. Initially, control with *S. riobrave* was quite variable but improvements were made as the system was understood better. By 1999, 19,000 ha of citrus were treated with *S. riobrave*.

A combination of several factors makes nematodes successful for this use. Soils of citrus groves are often quite sandy, thus facilitating nematode movement and oxygen availability; in fact, application of these nematodes to clay soils provides no control of citrus root weevil. These root weevils only occur within the drip line of trees so that they are protected from ultraviolet radiation and their environment remains moist. Therefore, nematodes only need to be applied in these specific areas. Probably most importantly, growers badly needed to control this pest and there was little to no competition with synthetic chemical pesticides in availability, price, or efficacy. With the limited area needing application, cost of nematode applications was low based on the high value of these orchards, resulting in widespread use of these nematodes.

Aside from *Steinernema* and *Heterorhabditis*, few other species have been considered for insect control. In recent years, *Phasmarhabditis hermaphrodita* has been developed for control of snails and slugs in the United Kingdom. Nematodes have some funky life cycles and this one lives as self-fertilizing hermaphrodites once it enters a new host and develops to the adult stage. *P. hermaphrodita* also uses the help of bacteria; it carries bacteria to a new snail or slug and then feeds on the bacteria that multiply within the host. Although it can take from 7 to 21 days for this nematode to kill a host, host feeding drops to only 10% of normal by 4 days after infection, thus preventing continued damage.

The mermithid *Romanomermis culicivorax*, attacking mosquito larvae, was studied extensively and found to provide effective control. Mass production was somewhat complex and expensive, but due to the high priority of mosquito control (Fig. 9.4), this mermithid was commercialized in the late 1970s and the product was named Skeeter Doom. However, the product predominantly failed due to storage and transportation problems in addition to being out-competed by a major new product on the market at that time, another natural enemy, the bacterium *Bacillus thuringiensis israelensis* (see Chapter 10).

FURTHER READING

- Bedding, R. R. & Akhurst, H. K. (eds). *Nematodes and the Biological Control of Insect Pests*. East Melbourne, Australia: CSIRO Publications, 1993.
- Gaugler, R. (ed.). *Entomopathogenic Nematology*. Wallingford, Oxon, UK: CABI Publishers, 2002.

Bacterial pathogens of invertebrates

Bacteria are diverse unicellular organisms that have no internal membrane-bound organelles, making them prokaryotes. They range in size from less than one to several microns long and therefore cannot be seen with the naked eye. Bacteria are ubiquitous, being found in virtually any potential habitats. They use a great diversity of materials as nutrients and divide by fission. Some examples of bacterial associates of invertebrates would be the saprophytic bacteria that live externally on animals and commensals that live within the guts of unwitting hosts, deriving protection from this association.

Some species of bacteria are more integrally involved with invertebrates, living as obligate symbionts within them. For example, bacteria in the genus *Buchnera* live within specialized host-produced cells, called mycetocytes, within aphids. These bacteria have lived inside aphids for so long that they have few morphological characters that can be used for identification. However, phylogenetic studies using molecular techniques suggest that the association between *Buchnera* and aphids began 160–280 million years ago (Douglas, 1998). This association is required by the bacteria and the aphids; aphids without *Buchnera* grow poorly because they depend on these bacteria for essential amino acids and *Buchnera* cannot be grown outside the aphids.

Pathogens are parasites that are microorganisms and some bacteria have adopted the life history strategy of living as pathogens of invertebrates. Pathogens often cause disease, a term simply meaning an unhealthy state. There are relatively few different species of bacteria specializing in this life strategy. Numerous species of bacteria are opportunistic and can overcome insects readily if they can gain entry to the body, as through wounds. However, most bacteria that are pathogens of invertebrates must be eaten by hosts and they then enter the body cavity through the gut. Once inside the gut, most bacteria are not able to simply enter the hemocoel directly, although some virulent pathogens have devised ways to breach the gut wall rapidly. Some species live more as obligate parasites and require a long time, sometimes even more than a month, to kill hosts while several of the more virulent species use toxins to damage the gut

wall and kill hosts quickly. When insects are infected with bacterial pathogens, their bodies can turn colors from white to red, amber, black, or brown. Cadavers from recently killed insects can be flaccid and fragile but, as the body dries, it often shrivels and becomes hard. In fact, the bodies of insects killed in any way make excellent media for growth of saprophytic bacteria. Therefore, cadavers of any dead insects will soon be colonized by microorganisms, especially including bacteria, making diagnosis of the cause of death due to bacteria more difficult.

The bacteria most widely used for biological control are spore formers in the Family Bacillaceae. Diseases caused by spore-forming species differ significantly, with mortality of hosts ranging from a matter of days to months for different bacterial species. Learning more about the cause of naturally occurring mortality of pests led to the discovery of a type of invertebrate/bacterial pathogen activity not reported previously. The non-spore-forming bacterium *Serratia entomophila* was found during investigations of deaths of pestiferous pasture scarabs in New Zealand; this bacterial species has a unique activity, killing by blocking the guts of infected hosts (Jackson *et al.*, 1992).

10.1 | Use for pest control

Use of bacteria for pest control has focused on their application as biopesticides. Only four species of bacteria have been mass-produced and commercialized (Table 10.1) but one of these, *Bacillus thuringiensis*, is used for inundative release more than any other biological control agent.

10.1.1 *Bacillus thuringiensis* (Bt)

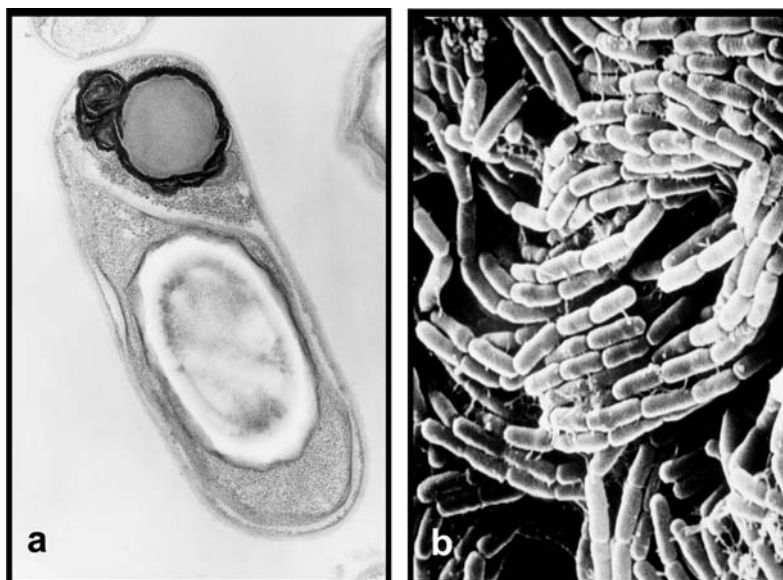
Bt is a rod-shaped soil bacterium that can be found worldwide on plants, in insects, and in soil, surviving in the environment as resistant spores. Interestingly, it is only rarely found causing epizootics in insect populations under natural conditions yet Bt has the power to kill many different kinds of insects and has been developed extensively for pest control in a variety of habitats, from field crops to controlling insect vectors of human disease, such as mosquitoes.

Bt is actually a complex of bacterial subspecies that are differentiated based on serology, with the commonality that all produce a spore as well as a parasporal body within a sporangium (Fig. 10.1). Parasporal bodies contain one or more proteinaceous protoxins in a crystalline structure and, therefore, these are frequently referred to as crystals. Crystals can account for 30% of the total protein content of the bacterial cell. Toxins in the crystals of Bt are called δ (delta)-endotoxins and subspecies of Bt have different δ -endotoxins that act on different hosts. There are also other toxins besides the δ -endotoxin that are specifically made by different Bt strains. A type of toxin that is excreted, a β -exotoxin, has been shown to pose some

Table 10.1 Species of bacteria mass produced for control of arthropods

Bacterial species	Target hosts	Mode of action	Speed of kill	<i>In vitro</i> mass production
<i>Paenibacillus popilliae</i>	Scarab larvae	Infectious disease	slow	—
<i>Bacillus sphaericus</i>	Mosquito larvae (espec. <i>Culex</i> and <i>Anopheles</i>)	Binary toxin in parasporal body	fast	+
<i>Bacillus thuringiensis</i>	Caterpillars, beetles, fly larvae	Toxin in parasporal body	fast	+
<i>Serratia entomophila</i>	Scarab larvae	Blocks gut	slow	+

Fig. 10.1 a. Sporangium of *Bacillus thuringiensis*; note the crystal above and spore below (Feitelson *et al.*, 1992).
b. Vegetative cells of *B. thuringiensis*. (Photo courtesy of Jean-Francois Charles, Institut Pasteur.)



risk to mammals and non-targets and thus care is taken that strains of Bt that have been developed for pest control do not produce this toxin.

When Bt is ingested by a susceptible host, the crystal is dissolved in the alkaline gut and the resulting protoxin is then cleaved by proteolytic enzymes in the gut to become activated (Fig. 10.2). Part of the toxin molecule attaches to the gut wall to form a pore. The formation of pores disrupts the osmotic balance across the midgut and gut cells subsequently swell and shrink and some eventually burst, allowing bacterial cells to invade the body of the host. The bacteria quickly proliferate within the compromised host and most susceptible species die within a day or two.

The isolates that were developed first for pest control are active against caterpillars (larvae of Lepidoptera), with the most commonly used subspecies being *B. t. kurstaki* (Btk) (Box 10.1). Lepidopteran species are differentially susceptible to the toxins, so finding individual strains of Bt that are active against different host species has been

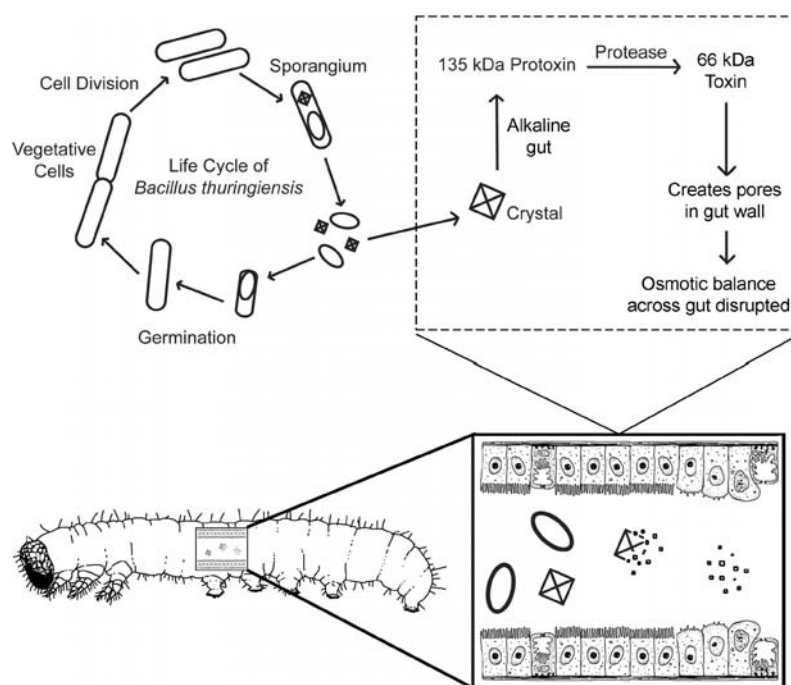


Fig. 10.2 Life cycle of *Bacillus thuringiensis kurstaki*. (After Tanada & Kaya, 1993.)

Box 10.1 | An unlikely experiment

In 1950, Dr. Edward Steinhaus, Director of the Laboratory of Insect Pathology at the University of California, Berkeley was conducting studies with viruses for control of alfalfa caterpillar, *Colias eurytheme* (Steinhaus, 1975). He was frustrated because the virus he was using was not killing the caterpillars fast enough to prevent damage to the crop adequately. One day he remembered that 8 years earlier, he had been sent a species of bacteria that was said to be a virulent pathogen. In fact, this strain of bacteria had been isolated from Mediterranean flour moth, a stored product pest, in the province of Thuringia in Germany and had been named *Bacillus thuringiensis*. Laboratory bioassays in Europe had suggested that this bacteria was a virulent pathogen against caterpillars and results from field trials against European corn borer had been promising (Tanada & Kaya, 1993).

Steinhaus took the long-forgotten bacteria out of the refrigerator and sprayed it on a tray of alfalfa plants hosting several hundred caterpillars (Steinhaus, 1975). When he came to work the next morning, most of the caterpillars had ceased feeding and were dying or already dead. Of course, Steinhaus was very excited but he knew that he needed to repeat this experiment before proceeding. He quickly began to grow more bacteria and, that evening, sprayed the freshly grown bacteria on a new batch of caterpillars. The next day, to his surprise, there was no effect on the treated caterpillars. There was the possibility that the spray equipment he had used initially had been contaminated with synthetic chemical pesticides and had not been cleaned properly so the first batch of insects might have all died from pesticide poisoning. However, washing the spray equipment and spraying the old culture again did not alter the results. Why did the first batch of larvae die when the second did not?



Edward A. Steinhaus, the father of modern insect pathology. (Reproduced with permission of Elizabeth Davidson.)

Steinhaus was not one to be deterred and his further work with this bacterial species showed that when cultures of these bacteria had matured, they formed spores and it was only at this point that they were insecticidal. With the spore is the proteinaceous crystal (or parasporal body) and presence of this crystal had been documented by visual examination. However, the function of the crystal was not understood and it was not until 1955 that it was proved that the parasporal crystals were the cause of toxicity. Freshly growing cultures with plenty of nutrients lack spores and crystals and thus would have no effect on caterpillars. The old cultures Steinhaus used initially certainly consisted primarily of spores and we know now that this is why they were therefore highly toxic. The young cultures he assayed second would have contained no spores and crystals and this explains their lack of effect on the caterpillars.

This was only the beginning of Steinhaus' work with *B. thuringiensis*. He went on to conduct basic and applied studies that demonstrated to both insect pathologists and industry the potential uses for this bacterium, which has grown to be the most frequently used type of biological control.

essential for targeting a diversity of pests. Dipteran-active strains were next to be found, in 1976, with the discovery of *B. t. israelensis* (Bti) infecting mosquitoes in Israel (Goldberg & Margalit, 1977). Bti products are now available for control of mosquitoes and blackflies that vector vertebrate diseases as well as nuisance species. In 1983, Krieg *et al.* reported the first Bt isolate active against beetles, *B. t. "tenebrionis"* (Btt; due to a name change, the proper name is *B. t. morrisoni*), and thus began development of Bt for control of beetle pests. The search for new strains of Bt continues and now strains active against Hymenoptera, Hemiptera, Mallophaga, Nematoda, and protozoa have been discovered. Due to intensive prospecting for strains with novel

activities, it has been estimated that at least 60,000 strains of Bt are held in collections around the world. These strains produce at least 25 different, but related, crystal toxins (abbreviated as Cry) and genes encoding many of these toxins have been sequenced. At present, 60 subspecies of Bt have been named but there are many strains having different activities available within each subspecies and new strains are found constantly (Federici, 1999). The Bt insecticidal proteins are highly specific toxins, active in insect guts, and thus they have a superior safety record with regard to non-target organisms.

Products based on the bacterial cells are generally composed of bacterial cells that had produced spores and crystals and were then lysed. Thus, usually the parasporal bodies containing the δ -endotoxins have already been released from the sporangia but spores are present too. A variety of formulations of Bt are available, including emulsifiable concentrates, wettable powders, and granules for use in many different habitats against many different pests. As examples, Bt sprays have been extensively used to control caterpillars in forestry, such as gypsy moth and spruce budworm in the northeastern United States. Also targeted are a variety of lepidopteran pests of crops and horticulture. An important use of Bti has been its application for control of mosquitoes and blackflies (Box 10.2). Beetle-active strains of Bt have been used to control Colorado potato beetle and leaf beetles in eucalyptus.

The ability to easily grow Bt in large quantities, such as 50,000 liter batches, makes this bacterium easy and cheap to mass produce. It is

Box 10.2 | Onchocerciasis and Bti (Becker, 2000)

Onchocerciasis, also called river blindness, is a human disease caused by filarial worms. The adult worms live in nodules under human skin and it is the immature stages that invade the eye where they damage tissues and then die. Humans do not die from this disease but the scarring in their eyes leads to blindness. These worms rely on adult blackflies to be transported to new human hosts.

The immature stages of blackflies live in flowing water so this disease affects many people living along rivers in some tropical countries. River blindness is predominantly a problem in fertile low-lying valleys along rivers in West Africa and South America. From 1975, the chemical pesticide temephos was applied to 50,000 km of rivers in 11 West African countries, often from the air. By 1979, the first signs of resistance to temephos were seen in the blackflies and studies with Bti began in earnest. By about 1985, an integrated program had been developed to use both Bti and temephos. Temephos continued to be used where resistance had not developed but by 1988, 81% of the region was protected by Bti. Substituting Bti for temephos presented some difficulties because Bti had to be applied more carefully, covering the entire river. Tanks and nozzles of application equipment could not be rusty and if the algal densities in the river were high, the Bti dose had to be increased. Overall, since this control program began, few new cases of river blindness have been recorded, thus protecting millions of people living in areas where this disease is endemic.

considered extremely safe and thus widely accepted by users. It can be stored indefinitely and can be applied using the same equipment and techniques as synthetic commercial insecticides at a reasonable cost. As of 1983, there were 410 registered formulations of Bt. In 1999 it was estimated that Bt is applied as a bacterial insecticide to millions of hectares each year, leading to a yearly worldwide market of \$100–200 million.

Genetic engineering using Bt

As methods for manipulation of genes exploded in the 1980s, scientists learned that it was relatively simple to manipulate the genes encoding Bt toxins. These genes occur on plasmids within the bacterial cells and are therefore relatively easy to alter and move. Genes encoding Bt toxins have been manipulated either by inserting them in new strains of Bt or by inserting them into other species of bacteria. Of course, a major development has also been expression of Bt toxin genes within plants. The ultimate goals of this genetic engineering have been to increase stability in the activity of Bt and sometimes to expand the host range.

Transconjugant Bt products are based on adding plasmids bearing genes for additional toxins to bacterial cells. For example, with the product Foil[®], toxin-bearing plasmids from a coleopteran strain of Bt were introduced into a lepidopteran-active strain so that the resulting product is active against both caterpillars and beetles (Baum *et al.*, 1999). For recombinant Bt products, Bt strains are usually engineered to include genes for overproduction of specific toxins and production of combinations of toxins in the same cells. To increase environmental stability by providing protection from ultraviolet light, recombinant plasmids were inserted into cells of the thick-walled bacterium *Pseudomonas fluorescens*. The *P. fluorescens* cells produce and then contain the toxin and are then killed before application. Thus, the toxin could be applied to the field within a thicker bacterial wall and thus retain activity longer.

The first Bt-modified transgenic plants were developed in the mid-1980s and this technology quickly expanded in the USA, where transgenic cotton was first sold in 1996, closely followed by corn and potatoes, and research and development continues with additional crops. However, the cost of developing Bt-engineered plants will probably limit the number of plant species developed in this way. To recoup their profits, companies selling seed of Bt-engineered plants require that farmers do not keep seed from transgenic crops that they grow. This new technology was rapidly adopted by US farmers because, although the seeds are costly, the resulting pest control was terrific, especially for combating pests living in concealed locations within plants that have been difficult to control using pesticides. During 1998 alone, c. 12 million acres of Bt corn and 2.8 million acres of Bt cotton were grown in the USA. Development of this new technology has not been without its critics. In fact, in the year 2000, farmers in Europe did not use transgenic crops at all and were vocally opposed to this technology, maintaining that side-effects of use of transgenic

crops have not yet been researched adequately. It seems this opinion is changing and use of this new technology is beginning to be considered in European countries.

Development of resistance

Researchers have worried that widespread use of Bt would lead to the development of resistance, just as resistance has developed to many synthetic chemical insecticides. Usually, development of resistance is not a concern for biological control agents. However, in some ways the activity of Bt is similar to synthetic chemical insecticides because its activity is often based on the activity of one chemical, the toxin. It was hypothesized that with heavy use, resistance to a Bt toxin could develop. Laboratory studies using many different host species repeatedly demonstrated that with high doses resistance could develop. Then, resistance was found in the field in diamondback moth populations feeding on cabbage or related crops at numerous sites in Asia and the USA. These diamondback moth populations had been exposed year round to Bt, often at high doses and on a regular basis. Interestingly, this is still the only host species for which true resistance has been demonstrated in the field. Although mosquito populations in many areas have received heavy exposure for years, for example in the Rhine Valley of Germany, no resistance in field populations has been documented.

Concern over development of resistance only began to reach a crescendo after Bt-engineered plants began being planted. Before that time, the development of resistance in only diamondback moth was thought to be due to the relatively small amounts of Bt applied to other pests, especially in comparison with chemical pesticides. It was argued that entire fields of Bt-engineered plants created exceptionally intense selection pressure and would hasten the development of resistance.

Development of resistance is especially a concern in the USA for growers wanting to use less pesticides. Growers who market their produce as being free of chemical pesticides and who often rely on use of Bt for pest control also worry. If resistance to Bt develops, these growers would have fewer alternatives for pest control.

Management strategies that have been suggested to prevent or delay the development of resistance to Bt include use of multiple toxins within the same treatments, planting non-sprayed or non-Bt-expressing plants so that not all insects are exposed to Bt (i.e. providing refugia), using very high doses of Bt toxin, planting mixtures of normal and transgenic seeds, or rotating the toxin being used by using different toxins at different times. The strategy that has received the most support in the USA is a combination of providing refugia and high doses of Bt. Resistance is thought usually to be recessive. Therefore, if insects that are resistant mate with insects that are not resistant, the offspring will not be resistant. By providing pests with crop plants not expressing Bt near the fields of transgenics, insects that are not resistant will continue to be present in the area and,

should any resistant insects from the Bt usage areas survive the exposure to high levels of toxin, they would mate with the refuge insects and their offspring would not be resistant. As a caveat, the efficacy of this strategy is still under investigation but it is the best alternative known. In fact, to prevent the development of resistance, companies selling Bt-engineered plant seeds require farmers to sign contracts stating that they will plant refuges near Bt transgenic crops to help try to prevent the development of resistance.

10.1.2 Fighting scarab grubs and mosquito wrigglers

Three more species of bacteria have been developed for control of invertebrates. *Bacillus sphaericus*, used against mosquito larvae (or wrigglers), actually has two different toxin proteins that are produced within each bacterial cell and both must be present for toxicity. *B. sphaericus* products are complementary to Bti because *B. sphaericus* has different uses. This bacterium survives better in more polluted water than Bti and it targets mosquito species that Bti is not effective against, such as *Culex*. *Culex* species are thought to be the major vectors of the human pathogen West Nile virus that was recently introduced to northeastern North America, and *B. sphaericus* is being used for control of vectors of this disease.

The spore-forming bacterium *Paenibacillus popilliae* was first found in the northeastern USA infecting larvae of the introduced scarab, Japanese beetle, in 1933. These grubs live in the soil and feed on grass roots. Larvae must eat the bacteria and, when infected, their hemolymph (blood) and the end of their body is milky-colored instead of clear, so the resulting disease was named “milky disease” (Fig. 10.3). After infection, this bacterium takes a long time to kill larvae unless the larvae are very young and ingest a large dose. *P. popilliae* is an obligate pathogen and thus cannot be easily grown outside

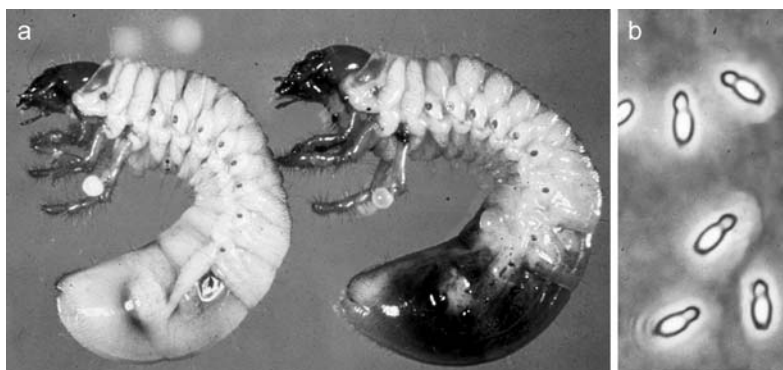


Fig. 10.3 a. Scarab beetle, *Rhopaea verreauxi*, grubs that are healthy (right) and infected with milky disease (left). Note milky appearance of the blood (hemolymph) seen through the abdomen and in droplet on leg (Photo courtesy of R. Milner). b. Sporangia of *P. popilliae*, often thought to look like shoe soles, with the crystals as the heels and spores as the soles. (Photo courtesy of M. Klein, USDA, ARS.)

the host insects. This has presented problems for mass-production of this bacterium. However, there are few ways to control such soil-dwelling pests and the lawns that Japanese beetle grubs damage are valuable. Therefore, this bacterium became one of the first insect pathogens developed as a microbial control agent in the USA. After this pathogen was discovered, huge programs were undertaken to distribute *P. popilliae*, releasing 109 tons of spore powder to over 90,000 sites over a 14-year period. The subsequent decline in Japanese beetle populations was attributed in part to activity of this pathogen. Because production of this obligate pathogen is difficult, it is expensive and not always available. As a side benefit, this pathogen persists well in the soil, being found 25–30 years after original applications, and thus can help with controlling Japanese beetle larvae over numerous years.

The grass grub *Costelytra zealandica* is a major pest of grasslands in New Zealand. In this case, the grasses have been introduced but this beetle species is native. A bacterial pathogen, *Serratia entomophila*, was found infecting these scarab grubs, which eventually turn amber when infected (thus, the disease is called “amber disease”). After ingestion, these bacteria block the gut and the scarab stops feeding within 24–48 hours, although it can take a long time before grubs actually die. The pathogen will often build up on its own when there are high populations of grubs but it can also be applied (inoculated) in pastures to promote early epizootics and prevent damage. Methods for mass-production in fermenters have been developed and this pathogen has been registered for use in New Zealand.

FURTHER READING

- Baum, J. A., Johnson, T. B. & Carlton, B. C. *Bacillus thuringiensis*, natural and recombinant bioinsecticide products. In *Biopesticides, Use and Delivery*, ed. F. R. Hall & J. J. Menn, pp. 189–209. Totowa, NJ: Humana Press, 1998.
- Charles, J.-F., Delécluse, A. & Nielsen-LeRoux, C. (eds). *Entomopathogenic Bacteria: From Laboratory to Field Application*. Dordrecht, NL: Kluwer Academic Publishers, 2000.
- Evans, H. F. (ed.). *Microbial Insecticides: Novelty or Necessity?* Farnham, Surrey, UK: British Crop Protection Council, 1997.
- Federici, B. *Bacillus thuringiensis* in biological control. In *Handbook of Biological Control*, ed. T. S. Bellows & T. W. Fisher, pp. 575–593. San Diego, CA: Academic Press, 1999.
- Glare, T. R. & O’Callaghan, M. *Bacillus thuringiensis: Biology, Ecology and Safety*. Chichester, UK: Wiley & Sons, 2000.
- Lacey, L. A., Frutos, R., Kaya, H. K. & Vail, P. Insect pathogens as biological control agents: do they have a future? *Biological Control*, **21** (2001), 230–248.
- Lacey, L. A. & Kaya, H. K. (ed.). *Field Manual of Techniques in Invertebrate Pathology*. Dordrecht, NL: Kluwer Academic Publishers, 2000.

Viral pathogens

In addition to bacteria, several other groups of microorganisms, including viruses, fungi, and microsporidia, also cause diseases in insects. Microorganisms utilize invertebrates for food just as they attack plants and other types of animals. Their relationships with hosts vary from obligate pathogens, which do not grow outside of the hosts in nature, to facultative pathogens, which only live as pathogens when an opportunity presents itself. The major microbial groups attacking invertebrates are roughly the same as those that have adopted life styles as pathogens of vertebrates and plants. As you know, virtually all species of pathogenic microorganisms infecting humans do not infect plants. Similarly, species of microbes causing disease in invertebrates generally specialize on invertebrates. The pathogens vectored by insects, such as malaria and plant pathogenic viruses, are special cases. In fact, within the invertebrates, pathogens display host specificity for certain groups and this is especially true of obligate pathogens that have close associations with hosts.

In some ways, pathogens of invertebrates have easier hosts to overcome than pathogens of vertebrates. The hard exterior cuticle of insects and mites poses a formidable barrier to microorganisms. If a pathogen enters the body of an insect or mite, these invertebrates then have an immune response for protection. However, invertebrate immune systems are quite different from vertebrate immune systems and are not as powerful. Numerous pathogens have developed the ability to overcome their invertebrate hosts and utilize the entire invertebrate body as a source of nutrients for microbial reproduction. Although some microbes can cause lingering, chronic infections, for control purposes the focus has been on microbes causing rapid death. In addition to the bacteria (Chapter 10), pathogens that can kill hosts relatively quickly include the viruses and fungi (Chapter 12). These pathogens affect a diversity of invertebrates but it should be noted that all microbes except fungi must be eaten in order to infect. Since fungi can penetrate directly through the cuticle, they are the pathogens infecting insects with piercing, sucking mouthparts, such as aphids, whiteflies, and scale insects. Viruses and fungi are especially known to cause dramatic epizootics in nature, so trying to

utilize this potential has been one driving force toward development of pathogens for pest control. A subgroup within the Protista, the microsporidia, are also naturally occurring pathogens of invertebrates and have occasionally been developed as control agents so this group will be mentioned briefly (see Chapter 12).

11.1 | General biology of viruses

Viruses are non-cellular genetic elements, containing either DNA or RNA, whose energy is derived from the host. Because viruses can only replicate themselves within a living cell, all viruses are obligate intracellular parasites. After they replicate their DNA or RNA genomes in host cells, viruses are then packaged into particles called virions that form the extracellular state that is infectious and is needed to reach new hosts.

Viruses are grouped based on their nucleic acid composition, their genome structure, and the morphology of their external coats. They are so small that the largest is barely visible with the light microscope. The largest viruses, the pox viruses, have virions up to 470 nanometers long. Thus, viral morphology must be investigated using the electron microscope and molecular biological techniques are a requirement for studying the activity of viruses. The basic structure of a virus is the viral DNA or RNA surrounded by a protein capsule and sometimes a membrane or envelope; this constitutes a virion.

Latin names are not used for naming species of viruses. Viruses are classified by family, and individual viruses are often named after the host or place in which they were first found, or sometimes they are named for the disease they cause, for example influenza virus, smallpox virus. As is common in all sectors of pathology, a disease, the negative impact of pathogen on a host, is often named before the causative pathogen is isolated and studied. Viruses have exploited a great diversity of hosts including vertebrates, invertebrates, plants, fungi, single-celled animals, and bacteria.

11.2 | Invertebrate viral pathogens

At least 13 viral families include pathogens of invertebrates (Hunter-Fujita *et al.*, 1998). Because they live within host cells and are therefore closely associated with hosts, many viruses are highly host specific. While some viruses attacking invertebrates occur in viral families that include viruses attacking vertebrates, the Family Baculoviridae is known only from insects and related invertebrates. Because of their pathogenicity and host specificity, viruses in the Baculoviridae are among the best-studied invertebrate viruses. These viruses are known to infect a variety of insects, but especially caterpillars (larvae of the Order Lepidoptera), sawflies (relatives of wasps and bees having immature stages very similar to caterpillars), and mosquito larvae. The

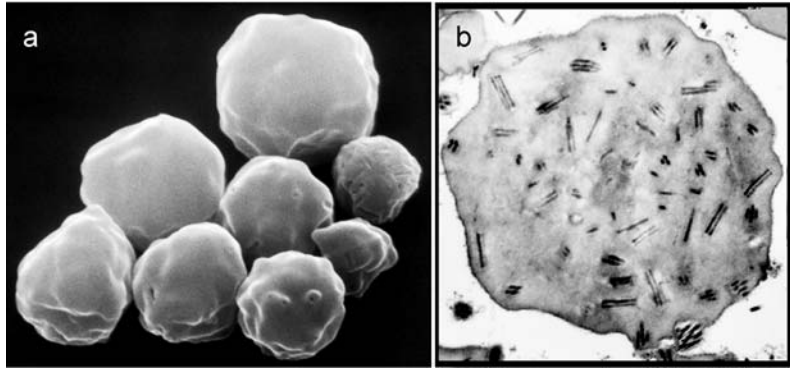


Fig. 11.1 a. Scanning electron micrograph of the occlusion bodies of a nuclear polyhedrosis virus. (Photo courtesy of J. Podgwaite, USDA, Forest Service.)
 b. Transmission electron micrograph of a cross section of an occlusion body from a nuclear polyhedrosis virus. The small dark structures within the protein matrix of the occlusion body are the virions. (Photo courtesy of James Slavicek, USDA, Forest Service.)

high degree of host specificity of most viruses makes them highly acceptable for numerous biological control purposes.

Among the viruses attacking insects, viruses in three families have a special adaptation for survival in the environment. Invertebrate viruses in the families Baculoviridae, Poxviridae, and Reoviridae produce an occlusion body (OB), a structure that protects virus particles or virions (Fig. 11.1). The occlusion body is resistant to environmental insults and could be considered analogous to a bacterial spore. For the baculoviruses, cytoplasmic polyhedrosis viruses (reoviruses), and pox viruses, occlusion bodies are made of a protein matrix in which from one to many of the infectious virions are embedded. Occlusion bodies are produced within infected invertebrates and are released into the environment after host death. Unprotected virions are fragile and die when desiccated or exposed to sunlight. The proteinaceous occlusion body protects the virions in the environment before they infect another host, thus enhancing viral survival both within a season and for the longer term, between seasons or for many years. Occlusion bodies vary in size and shape for different groups. Within the Baculoviridae, the nuclear polyhedrosis viruses (NPVs) have many-sided occlusion bodies (c. 0.5–15 μm) that can contain many virions (Fig. 11.1) while granulosis viruses (GVs) have smaller, capsule-shaped occlusion bodies (c. 200 \times 600 nm) that each contain one virion.

While most vertebrate viruses spread themselves from animal to animal by direct contact of a virus particle with a mucous membrane, viruses of arthropods generally must be eaten and they then infect through the gut wall. Because we know the most about the Baculoviridae and this is the main group that is being exploited for biological control, species in this family will be used as examples of how viruses interact with arthropod hosts. When occlusion bodies are eaten by a caterpillar, alkaline conditions within the gut can cause the protein

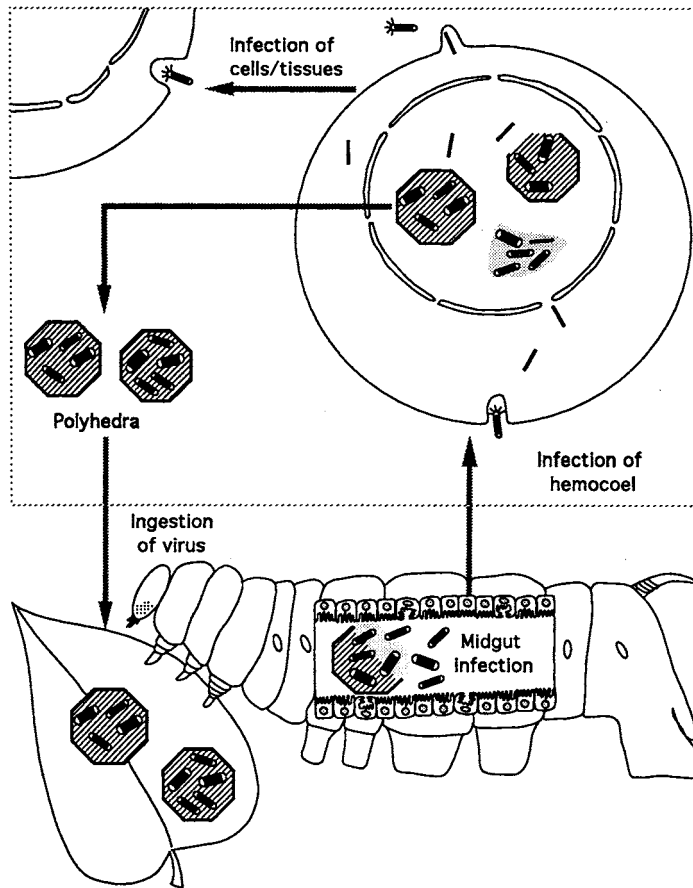


Fig. 11.2 The cycle of a baculovirus infection. The occlusion bodies (polyhedra) are ingested and the virus enters the midgut cells and replicates during primary infection. Non-occluded forms of the virus are then released into the hemocoel and these spread to infect further cells within the host. In later stages of the infection, the occluded form of the virus is produced and released. (Shuler *et al.*, 1995.)

matrix of the occlusion body to dissolve, releasing the virions within (Fig. 11.2). The outer layer of a virion then binds to and fuses with the cells lining the midgut of the host, and the viral particle enters the midgut cell. The virus then replicates within the nucleus of that cell and progeny viruses are produced to spread infection throughout the host. While some viruses of insects can cause more chronic diseases, the baculoviruses are often virulent pathogens that kill hosts relatively quickly.

Some viruses are tissue specific and only attack certain tissues but many baculoviruses attacking lepidopteran larvae invade and appear to replicate in all tissues. By the time the host dies, the cadaver is filled with multitudes of occlusion bodies. Cadavers of dead baculovirus-killed caterpillars typically hang upside down, sometimes attached by the rear prolegs or in an inverted "V" (Fig. 11.3). Baculoviruses are known to cause production of enzymes that break down the cells, tissues, and inner cuticle of the host. Therefore, at death or afterwards, the cuticle is very thin and the contents of the cadaver are liquefied. Eventually, the outer cuticle ruptures and the occlusion bodies within are released.

Fig. 11.3 Velvetbean caterpillar, *Anticarsia gemmatilis*, killed by nuclear polyhedrosis virus and hanging from the foliage in a characteristic “inverted V”. (Photo courtesy of Flavio Moscardi, EMBRAPA.)



Viruses have no way to disperse on their own so how do they reach a new host? Wind and rain are both thought to be important in dispersal of viruses. For baculoviruses infecting caterpillars and sawflies, the pathogen is transmitted quickly for hosts whose larvae feed gregariously. Occlusion bodies can be released when hosts defecate or when they die and their cuticles rupture. Occlusion bodies are then dispersed by being blown or washed from leaf to leaf, thus distributing the inoculum over a greater area. However, viruses also have some specialized methods for aiding dispersal. In Germany in the late 1800s, it was noted that before they died, some caterpillars of the nun moth, *Lymantria monacha*, climbed to the tops of spruce trees. This host behavior has clear advantages for the virus: when caterpillars die and the cadavers subsequently break open, virions are widely dispersed, being washed down onto lower foliage throughout the tree. We still do not understand the interactions between host and virus that cause such behavior but climbing before death has been seen with many other species of baculovirus-infected caterpillars. As a more exact method for dispersal, viruses can be picked up as hitchhikers when a parasitoid oviposits into an infected host and then can be inoculated into a healthy host the next time the parasitoid oviposits. Another major mode of baculovirus dispersal is by birds and small mammals that feed on infected insect larvae.

11.2.1 Use for pest control

Viruses have been used for long- as well as short-term insect pest control. With few exceptions, baculoviruses are the major virus group that has been developed, with particular emphasis on baculoviruses that produce occlusion bodies containing many virions, the NPVs.

Classical biological control

Many baculoviruses are known to cause epizootics in nature, so they have been utilized for classical biological control programs with highly successful results. Compared with parasitoids and predators, pathogens have not been used frequently for classical biological control. However, among the few instances where they have been used, there have been some stunning successes using viruses. The European spruce sawfly was permanently controlled through introduction of an NPV (Box 11.1).

A non-occluded invertebrate virus was successfully used to control the coconut palm rhinoceros beetle. These large beetles are major pests of coconut and oil palms in the South Pacific and Southeast Asia. The adult beetle bores into the heart of the palm tree, and severe infestations can lead to death of palms. The larvae develop in the decaying palm or in other decaying vegetable matter such as compost. Originally found in Malaysia, a non-occluded virus that principally develops in the gut cells of larvae and adults was found to be a potent biological control agent. The primary impact of this virus on

Box 11.1 | Introducing a virus against European spruce sawfly

The European spruce sawfly, *Gilpinia hercyniae*, was introduced to eastern Canada from northern Europe during the early 1900s and outbreak populations of larvae that are similar to caterpillars caused extensive damage. In 1936, a nuclear polyhedrosis virus was first observed attacking sawfly larvae, most probably accidentally introduced along with predators and parasitoids being introduced as part of a classical biological control program. In 1938, the sawfly occurred across 31,000 km² of forest but epizootics caused by this virus were first recorded. The virus spread rapidly on its own, increasing and causing epizootics as it spread. By 1943, the European spruce sawfly was no longer considered a pest. Extensive outbreaks of this sawfly species have not naturally recurred and this pest remains under control due to a combination of this virus (accounting for more than 90% of control) and the introduced parasitoids.

The great success of this virus has been attributed to its multiple methods for survival and transmission. This nuclear polyhedrosis virus differs from many NPVs infecting caterpillars because only the cells lining the host gut become infected. Within 48 hours of infection, viral occlusion bodies are released from the gut wall and pass into the environment when larvae defecate, thus spreading the pathogen. If an older larva is not killed by the virus, development continues but the resulting adult has a virus-infected gut and continues to disseminate the virus. Viral occlusion bodies have been found in bird feces throughout the year, acquired when birds fed on virus-infected or virus-killed sawfly larvae. Virus is also transmitted through external contamination of eggs laid by infected adult females. The virus persists in areas where epizootics have occurred because viral occlusion bodies are abundant in the surface layers of soil where the sawflies overwinter before becoming adults in spring.

the insect host is that infected adults do not live as long and infected females have reduced fecundity (Zelazny *et al.*, 1992). Although the non-occluded virions of this virus do not survive well in the environment, they are aided by the beetles in dispersal and transmission. Adults spread the virus when they mate and when they defecate in feeding galleries or breeding sites. When eggs hatch and larvae eat the virus deposited by adults, they become infected. Because this virus is less stable in the environment, researchers found that it is best released by collecting adult beetles, infecting them and then releasing them to disseminate the virus (Hunter-Fujita *et al.*, 1998). This virus was released on many islands where the beetle had been accidentally introduced. As an example, in the Fijian Islands, palm frond damage declined for 24–30 months after virus introduction, with low damage for at least 24 more months. However, it was found that in areas where concentrations of breeding sites were present, beetle outbreaks could reoccur and the virus then needed to be inoculatively released again. Based on the successes in classical biological control using baculoviruses and other viruses against arthropods, and the high degree of specificity characteristic of these viruses, use of viruses for classical biological control should be explored further in the future.

Inundative releases

The principal development of baculoviruses has been for use in inundative releases. While viruses can be applied with the same spray equipment as chemical pesticides, they do not kill immediately, as do chemical pesticides, or even as quickly as Bt. However, baculoviruses are valued because most are more host specific than Bt. Insects infected with baculoviruses may take 5–9 days before dying from an infection. Therefore, viruses are appropriate for maintaining host populations at lower levels but generally not for rapidly controlling very large pest outbreaks requiring immediate control. Insect control through mass application of viruses for inundative augmentation has been developed quite extensively around the world (Table 11.1). Efforts have predominantly focused on use of baculoviruses for control of foliar-feeding lepidopteran larvae. Products are usually wettable powders or liquid concentrates and can therefore be applied using methods similar to those used for chemical insecticides. Applications of viruses can be calculated based on larval equivalents (LE, the average number of occlusion bodies from a single cadaver) per hectare or the number of occlusion bodies per hectare.

At present, large quantities of viruses are usually produced within their larval insect hosts. While many insect pathogenic viruses can be grown in cell culture, thus far, this type of production is not being used for any product being marketed. Therefore, for mass-production of most insect pathogenic viruses, a host colony must be maintained. In developed countries, virus production has been restricted only to those systems with hosts that can be mass-produced on artificial diets. However, few baculoviruses are sold for pest control in developed

Table 11.1 | Worldwide research, development, and use of insect pathogenic viruses

	Number of viruses ¹		
	Field trials	Extension trials	Commercialization and use
North America	16	7	8
Central American and Caribbean	9	5	6
South America	13	5	4
Western Europe	17	12	6
Eastern Europe and former Soviet Union	15	8	10
Indian subcontinent	6	3	1
SE Asian and western Pacific	8	4	2
China and Japan	17	13	5
Africa	11	6	4
Australasia	10	5	1

¹ Number of viruses includes cases ranging from some work to development mostly complete.

From Entwistle, 1998.

countries due to (1) the limited markets due to the host specificity of most viruses, (2) the short half-life of the virus in the field, (3) the long time interval before pests die, and (4) the short shelf-life at elevated temperatures. There is now technology available to overcome the latter three impediments and work on baculoviruses for use in developed countries continues.

In developing countries, use of viruses for pest control has been much more successful. Mass-production of viruses is often a cottage industry or is done cooperatively by groups of farmers. The largest program for producing and applying viruses is the program for use of a baculovirus for control of the velvetbean caterpillar in Brazil (Box 11.2).

If a product is not mass-produced commercially, in some cases farmer training has enabled use of naturally occurring viruses for control. Farmers can collect cadavers of infected insects, store them in a refrigerator or freezer, then create a slurry and spray it on a crop at desired concentrations. For example, to control caterpillars of a large migratory hawk moth attacking cassava and rubber in Brazil, *Erynnis ello*, farmers use 20 ml of macerated cadavers of larvae killed by virus diluted with 200 liters water on each hectare. Virus must be applied against younger caterpillars and 90% mortality has been recorded within 4 days, with an estimated treatment cost of US\$2.00/ha (de Oliveira 1998).

Box 11.2 | Spraying virus to control velvetbean caterpillar in Brazil

Velvetbean caterpillar is a major pest of soybean in Brazil. Programs to utilize NPV for control of velvetbean caterpillar were initiated in the early 1980s in Brazil. By the mid-1990s, this NPV was applied yearly to 1 million hectares of soybeans (Moscardi, 1999). Much of the virus that is used is produced in the field, and, by 1999, 35 metric tons of NPV-infected velvetbean caterpillars were being produced each year (Fig. 11.3). A federal agency oversees virus production and awards contracts for production to five private companies. The virus is applied at 1.5×10^{11} occlusion bodies per hectare (cadavers from 50 infected larvae per hectare) once per season and it is rare that more than one application is needed per growing season. This virus costs farmers US\$0.75 per hectare, based on the price in 2003, although this can change based on pest populations and virus availability. Therefore, the cost of virus for velvetbean caterpillar control is lower than the cost of synthetic chemical insecticides. Farmers can also harvest their own virus after spraying the government-regulated material. Farmers are encouraged to spray virus when the velvetbean caterpillar population is not greater than 40 larvae of under less than 1.5 cm in length per ground cloth sample.

This program is effective because soybeans can tolerate some damage without decreases in yield, labor costs are low for the labor-intensive virus production, and the virus only needs to be applied once each year. With field production of the virus, yields each soybean season are variable, and production levels have ranged from 650,000 to 1,750,000 hectare equivalents over a 7-year period. Brazilian researchers are working on developing more efficient ways to produce this virus in the laboratory.

Genetically improved viruses

Efforts have been made to improve baculoviruses for use in control. Viruses have relatively small genomes that have been completely sequenced for some species and this provides a wealth of information for modifying viruses for agricultural applications. Emphasis to date has been on engineering viruses to increase speed of kill, thus decreasing insect damage. Different genes encoding invertebrate toxins, insect neurohormones, and enzymes have been engineered into NPVs. In particular, use of insect-specific toxins from scorpions and a mite have been shown to decrease time to death in the laboratory.

Viruses may also be modified so that they become less fit as viruses, but better suited to agricultural applications. For example, NPVs encode a gene producing an enzymatic protein (EGT) that inhibits the molting process of its host. Because molting is inhibited, immature insects feed longer and grow bigger, thus producing larger insects in which the virus could produce more viral progeny before hosts die. Thus, this gene confers a positive trait for the virus in its natural setting. When the EGT gene has been deleted, laboratory studies showed that NPV-infected caterpillars fed less and died more quickly. Such an engineered virus is less fit in nature because fewer progeny virions are produced but more useful for pest control because

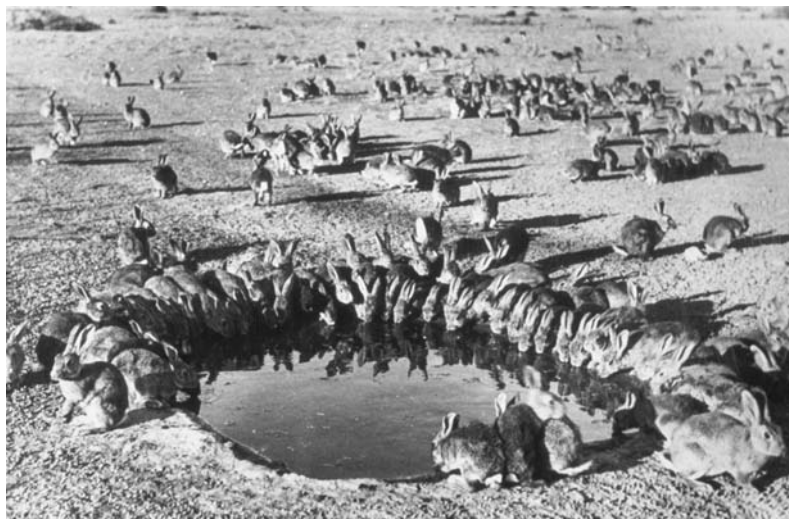


Fig. 11.4 European rabbits at an enclosed waterhole in South Australia in 1938. The abundant rabbit population destroyed pasture due to plant consumption and burrowing. (Photo courtesy of CSIRO.)

pests die more quickly. To date, field trials have been limited and no transgenic baculoviruses have been commercialized. For more extensive commercialization in industrialized countries, more efficient mass-production must be developed as well as methods for enhancing survival of occlusion bodies after they have been sprayed.

11.3 | Vertebrate viral pathogens

The most extensive biological control program directed against vertebrate pests has been the use of viruses to control rabbits introduced to Australia. Rabbits are not native to Australia but, in 1859, European rabbits were purposefully introduced to create a more home-like environment for European settlers. There were no natural predators of rabbits in Australia so rabbits rapidly increased and spread, becoming the most important agricultural pest (Fig. 11.4). Their feeding and burrowing destroyed pastures as well as causing erosion in the semi-desert interior.

11.3.1 Myxomatosis

In South America, a rabbit species closely related to the European rabbit (*Oryctolagus cuniculus*) was known to be infected by a pox virus called myxoma virus, which caused small benign fibrous tumors that persisted for months but were not fatal (Fenner & Fantini, 1999). While this disease, called myxomatosis, is not virulent toward South American rabbits, the European rabbit species that had been introduced to Australia was extremely susceptible and few individuals survived more than 13 days after infection. This pathogen is specific to rabbits and has no effect on humans; to prove to the public that this virus was safe for release in Australia, researchers went as far as injecting themselves with the virus and publicizing the lack of any effects from the injections.

Table 11.2 The virulence of strains of the myxoma virus isolated from Australian wild rabbits and tested by inoculating laboratory rabbits					
Virulence grade	I	II	III	IV	V
Degree of virulence	Extreme	Very high	Moderate	Low	Very low
Mean survival time (days)	<13	13–16	17–28	29–50	—
Case-fatality rate	99.5%	95–99%	70–95%	50–70%	<50%
1950–51	>99				
1951–52	33	50	17	0	0
1952–53	4	13	74	9	0
1953–54	16	25	50	9	0
1954–55	16	16	42	26	0
1955–56	0	3	55	25	17

After Marshall & Fenner, 1960.

In May 1950, this South American virus was introduced to Australia and by December, hundreds of rabbits were found to be infected, dying, or dead many miles from release sites. During 1950–51, the disease spread, causing 99% infection in some places, with an overall reduction in rabbit populations of 75–95%. This pathogen is predominantly vectored by mosquitoes and fleas that carry it from infected to healthy hosts; the relationship between vector and microbe is passive and the microbe does not reproduce within the vector. Directly after release, mosquitoes were the major vectors. To improve disease transmission, European rabbit fleas were introduced to Australia in 1968 and more xeric-adapted Spanish rabbit fleas were introduced in 1993.

By 1952, only two years after the initial release, scientists began to see a change in the interactions between rabbits and the myxoma virus. Since the virus was spread when mosquitoes took a blood meal from a living infected host and then fed on a healthy host, if the disease killed hosts quickly, this allowed little time for mosquitoes to transmit the disease. Through natural selection, strains of virus evolved that killed fewer rabbits and rabbits that died lived longer (Table 11.2). These changes were beneficial to the virus, providing more time for it to be transmitted. In addition, over 7 years, resistance to the pathogen was detected and then increased in the rabbit populations (Fig. 11.5). Thus, the virulence of the virus decreased while the resistance of the rabbits increased. This story provides a well-documented example of coevolution between host and pathogen, within a relatively short time period. The occurrence of coevolution raised the question whether the myxoma virus was still effective in controlling the rabbits. To test this, rabbits in enclosures were immunized with either a weak strain of the virus or a virulent strain. Rabbit populations were then exposed to naturally occurring myxomatosis in the field. After two years, even with the genetic resistance from the immunizations, this disease was still important in

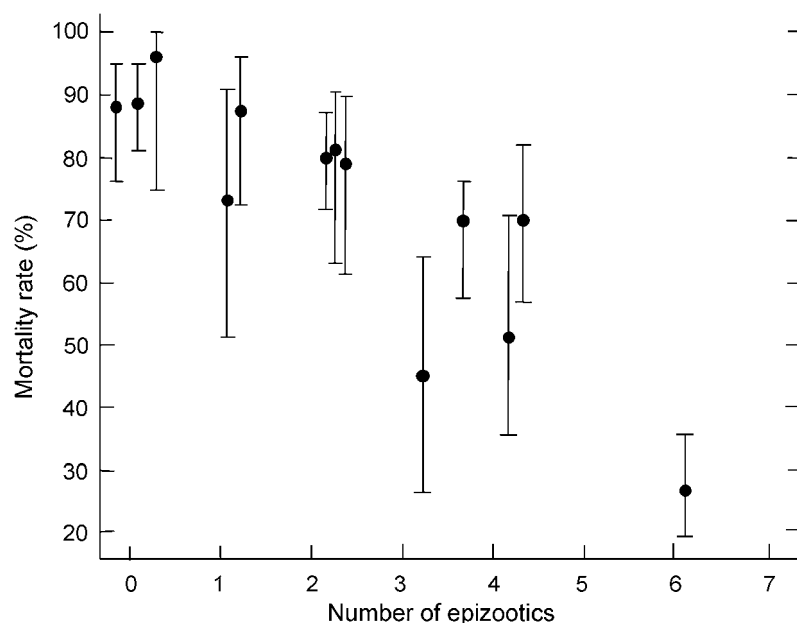


Fig. 11.5 Changes in the mortality rates of Australian wild rabbits exposed to varying numbers of myxomatosis epizootics. (Fenner & Myers, 1978.)

suppressing rabbit populations (Parer *et al.*, 1985), despite the reduced effect of the virus in the field.

In summary, after the changes in the rabbit/myxoma virus relationship, it was estimated that there were still 300 million European rabbits in Australia. While rabbits were not causing problems as catastrophic as before the virus was introduced, they once again had become major pests, causing an estimated Au\$115 million loss to wool and meat industries due to pasture destruction. In addition, habitats for native plants and animals were decimated, all due to introductions of rabbits by settlers wanting to make Australia seem more like Europe.

11.3.2 Rabbit calicivirus disease (RCD)

Due to the host/pathogen coevolution between European rabbits and myxoma virus in Australia, control due to myxomatosis was not complete. A new rabbit pathogen, a calicivirus, was discovered in angora rabbits exported from Germany to China. This calicivirus was very virulent, killing rabbits over 8 weeks old within 12–72 hours. Subsequent studies demonstrated that it was highly specific for only European rabbits. Australian scientists began investigations and in September 1995, this virus was accidentally established on the south coast of Australia when it escaped from a quarantine on an offshore island, possibly carried to the mainland by some combination of flies and winds. Rabbit calicivirus disease (RCD) immediately became established and began spreading and, between October and November 1995, an estimated 30 million rabbits in South Australia were killed by this disease. The majority of the surviving rabbits were younger than 6 weeks old. This disease spread fastest in spring and autumn at 10–18 km per day,

probably aided by humans, and is now considered well-established in Australia. RCD has been most effective in semi-arid rangeland where the rabbit problems were the worst. After RCD spread, in some areas there has been a noticeable regrowth of native vegetation, to a degree not seen within the memories of the present landholders.

FURTHER READING

- Evans, H. F. (ed.). *Microbial Insecticides: Novelty or Necessity?* Farnham, Surrey, UK: British Crop Protection Council, 1997.
- Federici, B. A perspective on pathogens as biological control agents for insect pests. In *Handbook of Biological Control*, ed. T. S. Bellows & T. W. Fisher, pp. 517–548. San Diego: Academic Press, 1999.
- Fenner, F. & Fantini, B. *Biological Control of Vertebrate Pests: The History of Myxomatosis – An Experiment in Evolution*. Wallingford, UK: CABI Publishing, 1999.
- Hoddle, M. S. Biological control of vertebrate pests. In *Handbook of Biological Control: Principles and Applications of Biological Control*, ed. T. S. Bellows & T. W. Fisher, pp. 955–975. San Diego: Academic Press, 1999.
- Hunter-Fujita, F. R., Entwistle, P. F., Evans, H. F. & Crook, N. E. *Insect Viruses and Pest Management*. Chichester, UK: Wiley, 1998.
- Lacey, L. A. & Kaya, H. K. (ed.). *Field Manual of Techniques in Invertebrate Pathology*. Dordrecht, NL: Kluwer Academic Publishers, 2000.
- Lacey, L. A., Frutos, R., Kaya, H. K. & Vail, P. Insect pathogens as biological control agents: Do they have a future? *Biological Control*, **21** (2001), 230–248.
- Miller, L. K. (ed.). *The Baculoviruses*. New York: Plenum Press, 1997.
- Treacy, M. F. Recombinant baculoviruses. In *Biopesticides: Use and Delivery*, ed. F. R. Hall & J. J. Menn, pp. 321–340. Totowa, NJ: Humana Press, 1999.

Chapter 12

Fungi and microsporidia

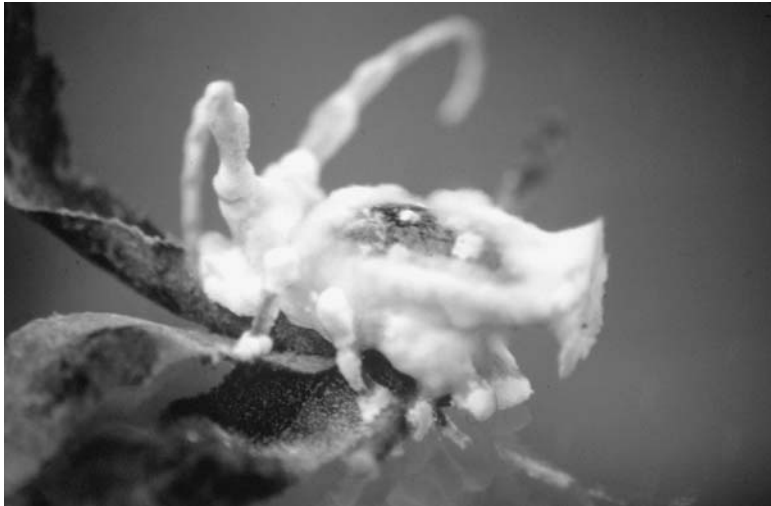
The remaining groups of invertebrate pathogens that have been exploited for biological control, the fungi and microsporidia, are eukaryotes. Although these groups share this more-advanced state of having nucleated cells, the fungi and microsporidia are vastly different groups.

12.1 Fungal pathogens of invertebrates

Fungi utilize a great diversity of resources as potential sources of nutrients but they need preformed organic matter for food. With this basic nutritional requirement, it seems logical that some fungi would have adopted life styles as pathogens. We will discuss fungi later as natural enemies attacking weeds (Chapter 15) and as both plant pathogens and antagonists of plant pathogens (Chapters 16 and 17). Although fungi are only very rarely lethal pathogens of vertebrates, they are important pathogens of many invertebrates. In fact, there are over 700 species of fungi that are pathogens of arthropods alone.

Among pathogens of arthropods, fungi are unique because most do not need to be eaten since they can penetrate directly through the cuticles of hosts. The microscopic reproductive units of fungi are called spores. First, a fungal spore must land on the surface of a potential host. If it can attach to the cuticle and is not inhibited, the spore will begin to grow and differentiate. The fungus can then penetrate the host cuticle, using both mechanical pressure and enzymes to digest the cuticle as it grows. Once within the hemocoel, the fungus increases, initially as single cells within the insect blood. For some fungi, the fungus proliferates in the host blood and eventually invades the host's organs and tissues shortly before the host dies. Other fungi kill the insect more quickly, possibly through use of toxins that they produce, and only utilize the entire cadaver after the host is dead. As with baculovirus infections, it generally takes several days for a fungus-infected host to die, with the length of time depending

Fig. 12.1 Cadaver of an adult Asian longhorned beetle, *Anoplophora glabripennis*, killed by *Beauveria brongniartii*. (Photo by A. E. Hajek; Hajek et al., 2001.)



on the fungal species and strain, the size of the host, and ambient conditions.

At some point in the process of infecting, utilizing, and killing hosts, the fungus switches over to growing as long tubes, or hyphae, a characteristic growth form for fungi. Generally, before an infected invertebrate host dies, there is little external evidence of fungal infection although the infected individual usually eats less. In some very interesting instances, as with some NPVs, host behavior can change and before death, the host climbs so that the resulting cadaver will be at an elevated location. In some cases, the fungus even grows out of the host and attaches it to the substrate before the host dies. Elevated locations for cadavers certainly improve the potential for dispersal of fungal spores.

Just after death of the host, cadavers are often somewhat hard in consistency. Under humid conditions, for most fungal pathogens, hyphae grow out through the host cuticle and spores are produced on modified hyphae outside the cadaver. The fungal growth on cadavers often appears fuzzy (Fig. 12.1) and this growth can be variously colored depending on the fungal species, often from white to greens to pinks, reds, and oranges. Spores are actively shot off from cadavers by some fungi while for other fungal species, spores are dislodged by wind or rain or just when contacted. Some fungal pathogens do not produce spores on the cadaver surface and instead, cadavers are filled with spores. As is typical of fungi, multitudes of spores are produced to increase the chances that a few might locate an appropriate host and successfully infect it. Fungal spores range from delicate short-lived structures that must remain wet to thick-walled “resting” spores that can survive for many years in the soil.

Fungi differ from other pathogens due to their ability to infect by penetrating the cuticle but there is a downside to this strategy. Fungal spores are more exposed to the external environment than

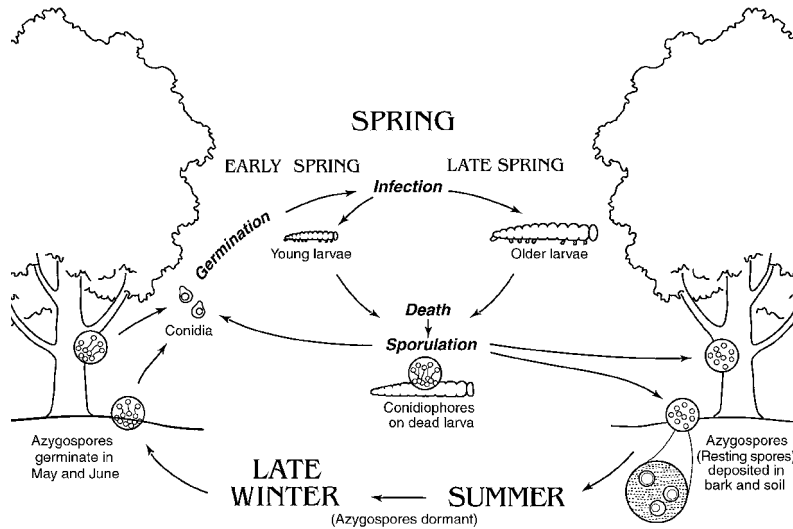


Fig. 12.2 Life cycle of the entomophthorean fungal pathogen *Entomophaga maimaiga* infecting gypsy moth, *Lymantria dispar*. (Illustration by Frances Fawcett.)

are pathogens that infect through the gut. Thus, these spores that require some moisture to germinate and infect could be exposed to potential desiccation. Fungal spores can also be exposed to ultraviolet radiation or high temperatures due to their external, exposed locations and this is risky because fungi can be sensitive to both of these environmental factors. While these limitations make infection sound unlikely, fungal pathogens of invertebrates are actually quite common in nature because they utilize the moist and cooler conditions found overnight and in localized microhabitats. In particular, fungal spores often persist in the soil through periods when hosts are not present or environmental conditions are unfavorable and thus the soil acts like a reservoir, storing fungal inoculum until infection is again possible.

12.1.1 Diversity of fungal pathogens

Two major groups of fungi having very different characteristics have been exploited for pest control. The Entomophthorales are obligate pathogens that are often quite host specific and have multiple spore stages that are either very short lived or very long lived. The long-lived resting spores provide a soil reservoir and short-lived asexual spores, or conidia, produced externally on cadavers are actively ejected to become windborne (Fig. 12.2). Using these specialized strategies, some Entomophthorales are able to respond to host populations and cause dramatic epizootics. Although most species of Entomophthorales can be grown outside of hosts, mass-production has been more difficult for this group. Due to the difficulties encountered with mass-production of Entomophthorales, strategies requiring use of small amounts of inoculum, that is, classical biological control or inoculative release, are most suitable for this group.

Fungi typically have complex life cycles with asexual and sexual stages but higher taxonomy is based on the sexual forms. However,

some groups are generally found only as the asexual forms and these are then grouped in the Deuteromycetes. For most members of the Deuteromycetes that attack arthropods, the sexual forms, when these can be found, belong to a large fungal group called the Ascomycota. The infective asexual spores (conidia) of many species in this group are somewhat more long lasting than the short-lived infective conidia of the Entomophthorales. Pathogens in this group are often able to grow outside hosts to some limited extent as saprophytes. These species are thus facultative saprophytes and, due to their more-flexible nutritional requirements, they are often more easily mass-produced.

While many fungi attack terrestrial hosts, there are also species attacking aquatic hosts and these have especially been investigated for control of mosquito larvae. One group historically considered within the fungi, the Chromista (previously Oomycetes), has recently been determined to be more closely related to the protozoa and algae. Spores of the chromist pathogen infecting mosquitoes, *Lagenidium giganteum*, have flagellae and can swim to locate hosts. Another mosquito pathogen, *Coelomomyces stegomyiae*, belongs to a more primitive group of fungi that also has flagellate spores. For some time, studies on *C. stegomyiae* were stymied because it could not be cultured in the laboratory. Researchers discovered that this pathogen has an obligate alternate host, a small aquatic crustacean (copepod); for a complete generation of this fungus, mosquitoes become infected followed by copepods, with different fungal forms associated with each host; the spores infecting mosquitoes cannot infect copepods and the spores infecting copepods cannot infect mosquitoes.

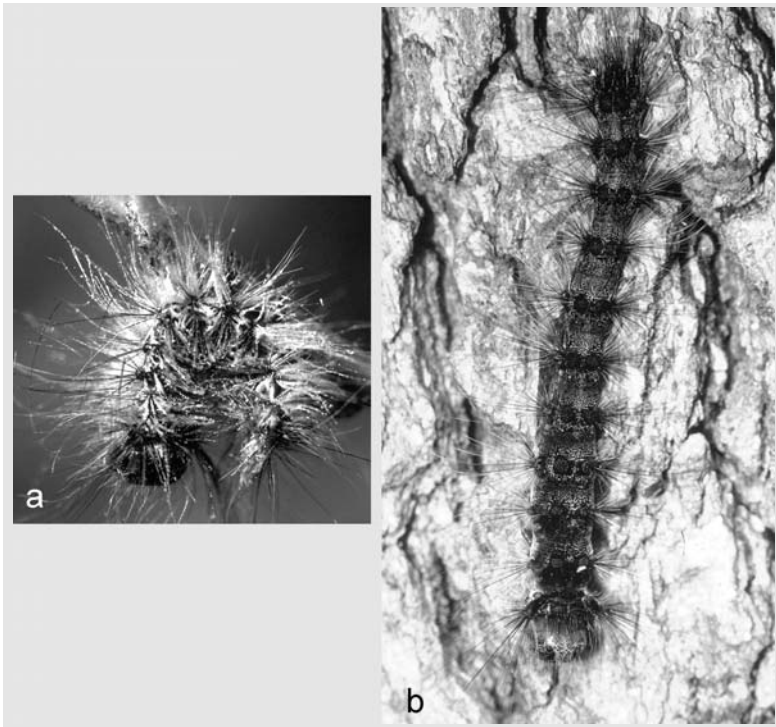
12.1.2 Use for pest control

Classical biological control

As with viruses, entomopathogenic fungi have not been used extensively for classical biological control. However, among the few attempts at classical biological control, the Entomophthorales have usually been used because these obligate pathogens can cause dramatic epizootics, responding to changes in host population density on their own. There have been some important successes in use of fungal pathogens for classical biological control. In one example, the dramatic epizootics characteristic of species of Entomophthorales have been put to use for control of gypsy moth by *Entomophaga maimaiga* (Box 12.1).

Box 12.1 | A fungus among us (Hajek, 1999)

Gypsy moth was accidentally introduced from France to northeastern North America in 1868 or 1869. In its new home, gypsy moth larvae fed on leaves of many species of trees, but especially oaks, and proceeded to increase in numbers and spread. Gypsy moth populations are still spreading today and are well known to increase to very large and damaging populations, called outbreak populations. During outbreaks, in springtime the large and hairy gypsy moth larvae become



a. Gypsy moth cadaver killed by *E. maimaiga* from which conidia have been actively ejected. Some conidia adhered to the hairs on the cadaver. b. Cadaver of older gypsy moth larva killed by *E. maimaiga* that is filled with resting spores, the overwintering stage of this fungus. (Photos courtesy of Donald Specker.)

less particular and will eat the leaves of almost all tree species in an area. In one record-breaking year, gypsy moth larvae defoliated trees across 13 million acres (5.3 million hectares) of forests in the northeastern USA.

This devastating species has been the target of biological control programs since the early 1900s. In 1910 and 1911, a fungal pathogen specific to gypsy moth was introduced to North America from Japan. However, researchers did not think it became established and no one ever saw it in the field. In 1989, populations of gypsy moth were on the increase but high levels of mortality were seen, with cadavers of large gypsy moth larvae hanging on tree trunks. These cadavers were filled with the resting spores of a fungus in the Entomophthorales, *Entomophaga maimaiga*. This is the same fungus that had been introduced from Japan in 1910–11 but no one had ever found an infected gypsy moth larva in the intervening years. We still don't know if the fungus released during the early introductions survived but required 79 years to establish and provide the control seen in 1989 or whether the fungus first found in 1989 was some more recent accidental introduction from Asia.

E. maimaiga has two types of spores, short-lived conidia that are actively ejected from cadavers of younger larvae and long-lived resting spores that remain within cadavers of larger larvae. Cadavers of younger larvae are found in tree and shrub canopies, where the conidia actively ejected from cadavers can readily become

airborne. Cadavers of larger larvae are found attached to tree trunks, eventually falling to the soil where they break open and resting spores are then washed into the soil at the bases of trees. Resting spores can persist for at least several years in soil at tree bases in large numbers, creating a reservoir of fungal inoculum so that this fungus is present and, given adequate environmental conditions, is able to increase when gypsy moth populations increase again.

Since 1989, *E. maimaiga* has repeatedly caused epizootics in gypsy moth populations that were at both high and low densities. Research has shown that this fungus requires moisture for spores to germinate and it seems that *E. maimaiga* is more active during northeastern springs with at least average levels of moisture. It seems that the activity of this fungus could be decreasing the frequency of gypsy moth outbreaks in the USA. If outbreaks do occur now that *E. maimaiga* is established in North America, it is possible that they will not be as severe or will not last as long. However, time is needed to collect the long-term data to test these hypotheses.

Inundative releases

Among the great diversity of species of fungi pathogenic to arthropods, only a handful of species have been developed for inundative release (Table 12.1). The vast majority of programs have utilized *Beauveria bassiana* and *Metarhizium anisopliae* or their close relatives. These fungi can be grown on inexpensive artificial media and have a relatively long shelf-life, especially if stored in the cold.

While few species have been exploited, this does not mean that they have been used little (Hajek *et al.*, 2001). Large programs have been developed for use of *B. bassiana* against pine caterpillars, *Dendrolimus* spp., and corn borers in China, where this fungus is economically competitive with synthetic chemical pesticides and is used on over 1 million hectares per year. Use of *M. anisopliae* against sugarcane spittlebugs, *Mahanarva posticata*, in Brazil has been on-going since the 1970s. This pest is well suited for control using *M. anisopliae*; although this fungus does not kill all of the spittlebugs, some spittlebugs can be present without significantly reducing sugarcane yield. Also, use of *M. anisopliae* is favored because this fungus is compatible with releases of hymenopteran and dipteran parasitoids against other pests on this crop, sugarcane borers. Parasitoids of sugarcane borers are very sensitive to chemical pesticides and use of *M. anisopliae* against spittlebugs on the same crop does not harm them.

Table 12.1 demonstrates that fungal pathogens are mass-produced for control of a diversity of pests, in fact pests from all of the major insect orders. For these products, usually conidia are applied by spraying them. Thus, survival of fungal spores is important and formulations to improve spore survival have been developed. One product based on *M. anisopliae* has been developed in Africa to control locusts (Box 12.2). Emphasis with this product has been on use of ultra-low volume application of formulations of conidia in oils. This facilitates application over large areas and helps the spores survive longer.

Table 12.1 | Some fungal pathogens registered or under commercial development for insect control around the world¹

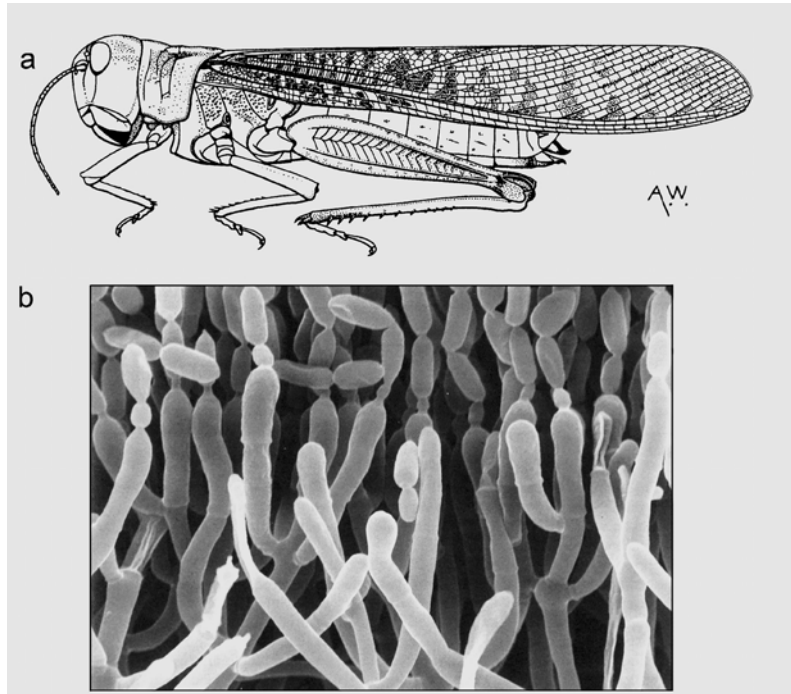
Fungal species	Group of host	Specific hosts
<i>Beauveria bassiana</i>	Coleoptera (beetles)	Cotton boll weevil (<i>Anthonomus grandis</i>), Colorado potato beetle, coffee berry borer (<i>Hypothenemus hampei</i>), pepper weevil (<i>Anthonomus eugenii</i>)
	Hemiptera (bugs, aphids, etc.)	Aphids, whiteflies
	Lepidoptera (moths)	Armyworms, European corn borer, pine caterpillars, codling moth, diamondback moth
	Orthoptera	Grasshoppers
	Thysanoptera	Thrips
<i>Beauveria brongniartii</i>	Coleoptera (beetles)	European cockchafer (<i>Melolontha melolontha</i>), white grubs, longhorned beetles
<i>Lagenidium giganteum</i>	Diptera (flies)	Mosquitoes
<i>Metarhizium anisopliae</i>	Isoptera	Termites
	Coleoptera (beetles)	Red-headed cockchafer (<i>Adoryphorus couloni</i>), black vine weevil
	Blattodea	Cockroaches
	Hemiptera (bugs, aphids, etc.)	Sugarcane and pasture spittlebugs
<i>M. anisopliae</i> var. <i>acridum</i>	Orthoptera	Grasshoppers, locusts
<i>Paecilomyces fumosoroseus</i>	Hemiptera (bugs, aphids, etc.)	Whiteflies
<i>Verticillium lecanii</i>	Hemiptera (bugs, aphids, etc.)	Aphids, whiteflies
	Thysanoptera	Thrips

¹ Products are variously being produced on all continents.

Hajek *et al.*, 2001.

Box 12.2 | Teeth of the wind (Lomer *et al.*, 2001)

Grasshoppers are one of the most conspicuous of insect pests and can become extremely abundant at times. About 20 species of grasshoppers are capable of aggregating in immense swarms that can migrate for sizeable distances and cause extensive damage to vegetation, of course without regard to crops or environmentally sensitive areas. Species with this particular life history are commonly called "locusts." One of the most famous species, the desert locust (*Schistocerca gregaria*) devastates crops across a wide swath of Africa at unpredictable intervals of approximately every 10–20 years. One of the largest swarms in the twentieth century covered about 1000 square kilometers and contained about 40 billion locusts. These locusts consumed vegetation equivalent to the food needs of 200 million people. In Arabic, the name for these overwhelming pests is the "teeth of the wind." The need to control such locust swarms is clear. However, application of highly toxic, broad spectrum chemical insecticides over such large and sensitive areas led to demands for alternative control measures.



a. The African locust, *Locusta migratoria* (Dirsh, 1965), only one among many species of pest grasshoppers in central Africa and worldwide. b. Hyphae and spores (conidia) of the fungus *Metarhizium anisopliae* var. *acridum*, which is mass-produced for locust and grasshopper control in Africa. (Milner, 1997.)

A hyphomycete fungal pathogen has been developed for control of locusts. One aspect of the aggregation behavior of locusts has been critical for development of fungi for locust control. Young nymphs aggregate in “bands” before they develop into adults that can fly and become the moveable, highly destructive swarms. The so-called “hopper bands” have been the main target in development of entomopathogenic fungi for inundative release. The most efficient method for application of fungi in remote rangelands and other roadless areas is via ultra-low-volume sprays from fixed wing aircraft. The droplets applied by concentrated ultra-low-volume sprays are very small and it is important that they do not evaporate before reaching the target. To prevent evaporation, oils were investigated as formulations for fungal spores and paraffinic oils were specifically found to be highly compatible with fungal spores. The fungal species found to be optimal against numerous species of locusts was *Metarhizium anisopliae* var. *acridum*. After an infected grasshopper has died, the hyphae growing from the cadaver are white but the cadaver soon appears fuzzy and green once the dark green spores are produced from hyphae. Locusts are known to bask in the sun and can increase their body temperatures enough to kill fungal pathogens growing within them. This fungal species was chosen, at least in part, because of its great ability to withstand high internal body temperatures 100–104 °F (38–40 °C), so that infected locusts could not easily cure themselves of fungal infections by basking.

The combination of *M. anisopliae* var. *acridum* formulated in oil and ultra-low-volume application can kill 70–90% of treated locusts in sparsely vegetated habitats, with no detectable impact on non-target organisms. This pathogen was developed for inundative release by a company in South Africa and the resulting product is named Green Muscle. It is sold as dry, unformulated conidia with a shelf life of at least 1 year at 30 °C and is mixed with oil for application. One drawback of this product is its slow speed of kill (10–20 days) and higher cost than synthetic chemical insecticides. However, Green Muscle provides the added benefit of so-called recycling in the field, meaning that once infected insects die, the fungus produces spores from the dead hosts and these spores can infect yet another group of insects. In addition, some of the spores initially sprayed that do not contact hosts remain viable in the field as a pathogen reservoir.

A few products utilize the behavior of hosts to make sure they become inoculated with spores. For example, cockroach traps using *M. anisopliae* were designed so that cockroaches entered and became inoculated with fungal spores when they hid within traps, standing with their backs contacting *M. anisopliae* conidia.

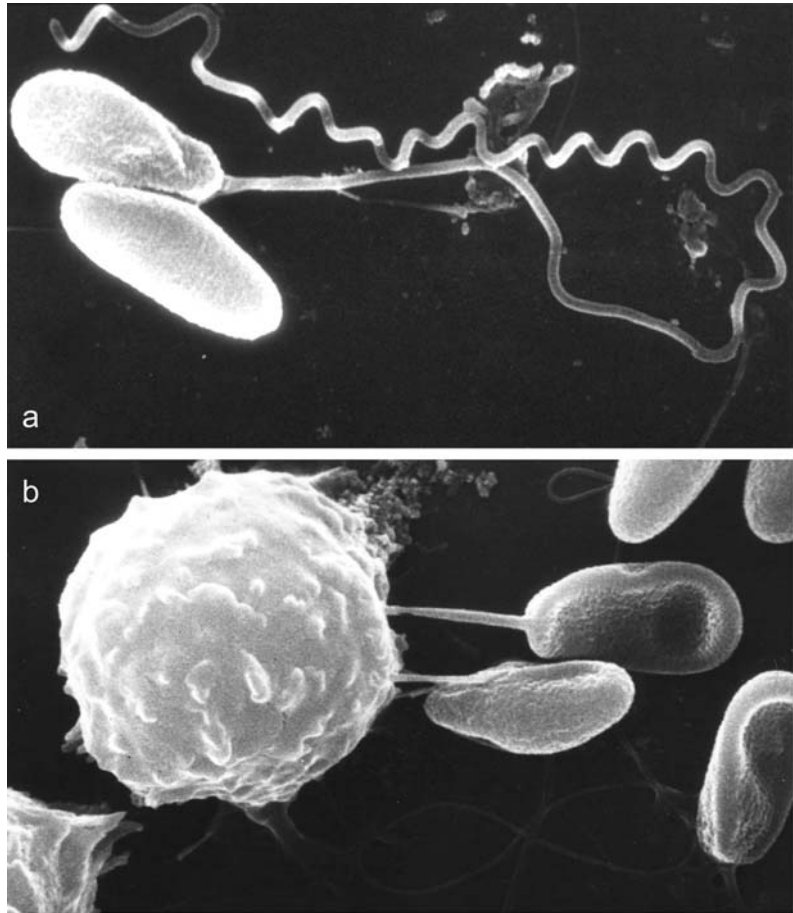
A Japanese product has been developed to control longhorned beetles whose larvae bore in the trunks of orchard trees. Adult longhorned beetles wander on tree trunks as they become sexually mature and they then mate and females lay their eggs under bark. During this time of wandering, adult beetles contact fiber bands containing fungal cultures that encircle tree trunks, thus becoming inoculated with spores. The cuticles of these beetles are thick and hard but the spores send hyphae to penetrate the thinner, bendable cuticle between the hard sclerotized plates of these adult beetles. The fungal cultures hanging on trees where beetles inoculate themselves can be covered with viable conidia for at least 30 days and can cause significant population reductions without frequent band replacement.

12.2 | Microsporidia

Although species from a diversity of groups of single-celled animals infect insects, the most common pathogens of invertebrates, and the only group investigated for use in pest control, are the microsporidia. Although microsporidia have historically been considered within the protozoa, based on recent molecular evidence they are now considered quite similar to fungi (van de Peer *et al.*, 2000). They are among the smallest eukaryotes and they live within the cells of hosts. Therefore, as with viruses, microsporidia always live as pathogens.

Microsporidia have fascinating and intricate life histories. Intracellular stages are often multinucleate, going through very complicated life cycles alternating between producing spores (most <6 µm long) and proliferating vegetatively. Microsporidian species often grow only in specific host tissues such as the fat storage organ (fat body), the

Fig. 12.3 a. Microsporidian spores, one of which has extruded its polar filament. b. Two microsporidian spores that have extruded their polar filaments, which have impaled a target cell. (Photos courtesy of Andreas Linde.)



midgut wall, or the reproductive tissues. After proliferation, microsporidia produce environmental spores that must be eaten by new hosts in order to infect. Much of the interior of these spores is filled with a long, coiled tube or polar filament. Once a spore is within the midgut of a potential host, the digestive juices of the host act on it to cause the polar filament to be extruded. This tube acts as an extensible inoculating needle, piercing a cell in the midgut wall as the contents of the spore pass through the tube to enter the host cell and begin a new infection (Fig. 12.3).

As a group, microsporidia infect a diversity of hosts, from vertebrates to various one-celled animals, but their most common hosts are arthropods. Most microsporidia cause chronic diseases that do not kill hosts and hosts often display few symptoms when infected. Infected arthropods often survive to adulthood but infected individuals are usually much smaller than normal and their reproductive output is often reduced. To ensure that microsporidia persist in low-density or scattered host populations, these pathogens often are passed from adult to offspring within the host eggs.

Although microsporidia often do not kill hosts, they can have an overall debilitating effect on host populations. They have been exploited for control very seldom, in part because they cause chronic infections and not host mortality but also because they must be produced in hosts. Some attempts at development for control have been made but the most long-standing has been use of the microsporidian *Nosema locustae*, developed for control of rangeland grasshoppers in the Great Plains of the USA (Henry & Oma, 1981). Because this species is an obligate pathogen, it must be produced in insects. The environmental spores produced in infected grasshoppers are harvested from cadavers and mixed with wheat bran, which acts as a bait to attract grasshoppers. When sprayed on a pasture, many grasshoppers became infected that same year. After releases of the spores plus bran, *N. locustae* persisted to the next year and the area with infected individuals expanded. It is thought that spread and persistence of *N. locustae* after release was due in part to passage of the microsporidia through host eggs to the next generation. It is also considered important to disease transmission that healthy grasshoppers often cannibalize diseased individuals or eat cadavers that they find, thus becoming infected.

FURTHER READING

- Becnel, J. J. & Andreadis, T. G. Microsporidia in insects. In *The Microsporidia and Microsporidiosis*, ed. M. Wittner & L. M. Weiss, pp. 447–501. Washington, DC: American Society for Microbiology, 1999.
- Brooks, W. M. Entomogenous protozoa. In *Handbook of Natural Pesticides. Vol. V, Microbial Insecticides, Part A, Entomogenous Protozoa and Fungi*, pp. 1–149. Boca Raton, FL: CRC Press, 1988.
- Butt, T. M., Jackson, C. W. & Magan, N. (eds). *Fungi as Biocontrol Agents: Progress, Problems and Potential*. Wallingford, UK: CABI Publishing, 2001.
- Evans, H. F. (ed). *Microbial Insecticides: Novelty or Necessity?* Farnham, Surrey, UK: British Crop Protection Council, 1997.
- Hajek, A. E., Wraight, S. P. & Vandenberg, J. D. Control of arthropods using pathogenic fungi. In *Bio-Exploitation of Fungi*, ed. S. B. Pointing & K. D. Hyde, pp. 309–347. Hong Kong: Fungal Diversity Press, 2001.
- Lacey, L. A., Frutos, R., Kaya, H. K. & Vail, P. Insect pathogens as biological control agents: do they have a future? *Biological Control*, **21** (2001), 230–248.
- Lacey, L. A. & Kaya, H. K. (ed.). *Field Manual of Techniques in Invertebrate Pathology*. Dordrecht, NL: Kluwer Academic Publishers, 2000.
- McCoy, C. W., Samson, R. A. & Boucias, D. G. Entomogenous fungi. In *Handbook of Natural Pesticides. Vol. V, Microbial Insecticides, Part A, Entomogenous Protozoa and Fungi*, pp. 151–243. Boca Raton, FL: CRC Press, 1988.

Part III

Biological control of weeds

Weeds are a major economic and environmental problem throughout the world and pesticides are regularly applied to control them. In the USA, it was estimated in 1980 that 500 major species of weedy plants were responsible for yearly losses of \$8 billion (Chandler, 1980). Herbicides to control weeds comprise 47% of the worldwide agrochemical sales while insecticides comprise only 29% (Woodburn, 1995). In the developing world, where herbicides are rarely used, removing weeds by hand can account for 60% of the pre-harvest labor input.

Weeds are not only a problem in agriculture. We consider any plant a weed that is undesired and grows in profusion, often out-competing desired plants. We often think first of weeds important to agriculture or horticulture, although weedy plants also impact natural areas. Non-native plants that are introduced to a new area often have a competitive advantage over natives because herbivory is absent or has a minor impact (the enemy release hypothesis). In such cases, invasive plant species outcompete native plants. Controlling invasive weeds that have spread to cover enormous areas such as pastures or wetlands is not feasible using direct controls such as synthetic chemical pesticides or cultural control. Aquatic weeds are especially difficult to control; herbicides must be added to water where they are quickly diluted and could later potentially be ingested by humans. Cultural control such as weed removal provides only a temporary solution. In such instances programs for biological control of weeds have produced some startling successes.

In agriculture and horticulture, plant species that typically are selected for use are easy to establish, grow rapidly, and are highly competitive, all characteristics leading to great potential for becoming an invasive weed. Introductions of new species of plants and trees for agriculture and horticulture have been long standing and plant introductions for forestry and pastures have added to this practice over the last few decades. Frequently, newly introduced species are

not considered weeds for up to 50 years, as they become established and spread (Hughes, 1995). Therefore, we are not yet seeing the potentially devastating effects of recent introductions. In Australia, 13% of plants imported for use in pastures have increased and spread to become weeds while in the United Kingdom, most destructive weeds were originally introduced as ornamentals for gardens. Most (85%) invasive woody plants in the United States were introduced as ornamentals. With the globalization of world resources, there is ever more potential for plants useful in one geographic area to be introduced to another where they could establish and spread to become weeds (see Chapter 1).

The impact of introduced weeds can be devastating to natural ecosystems and invasions by alien plants now threaten natural areas on all continents except Antarctica. Encroaching weeds can affect natural ecosystems directly by replacing the native vegetation. They can also affect the ecosystem indirectly by altering the environment enough so that native plants and animals can no longer live there. For example, when the weedy salt cedar tress, *Tamarix* spp., invaded a freshwater marsh in California, transpiration increased and the marsh was drained. To prevent alteration of natural areas by weeds, conservation scientists frequently turn to use of biological control.

Biological control of weeds has provided successful control of invasive weeds predominantly by introducing herbivorous insects. At present, other biological control strategies against weeds are minor in comparison with the classical biological control programs. However, efforts have diversified and to a limited extent plant pathogens have also been used for classical biological control. Plant pathogens have also been developed for inundative releases against agricultural weeds.

Biology and ecology of agents used for biological control of weeds

13.1 Types of agents

Plants are used by a great diversity of organisms that have different ways of exploiting them for food and shelter. The majority of herbivores that have been used for biological control of weeds are plant-feeding (phytophagous or herbivorous) invertebrates. These are often classified into guilds according to the plant resources they consume, such as roots, stems, leaves, flowers, seeds, fruits. Secondly, they can be considered by their style of feeding, such as sucking, chewing, leaf mining, stem mining, root boring. One specialized type of feeding is gall formation. Plant tissues can be attacked by invertebrates early in development and the plant is tricked into creating a specialized enlarged structure called a gall, which often becomes home plus food for invertebrates living within. Affected plants can expend lots of energy making galls, at the expense of other vegetative growth. If a gall is in the reproductive tissues, this leads to reduced seed output.

Where and when an invertebrate attacks a plant can have profoundly different effects and of course this also varies by plant species. Insects attacking the growing tips of plants can have a greater effect than insects solely feeding on leaves, although some plants can compensate for this damage and create new growing tips. Sap-sucking or gall-forming insects often are thought to have little effect, although they cause a prolonged drain on plant nutrients (but see section 14.3.1). Of course, fruit and flower feeders only affect plant reproduction and not the plants that are present.

Invertebrates feeding on plants rarely have an impact when feeding alone. Their effect is dependent on many individuals being present and active. Factors negatively affecting herbivore density such as predators, parasitoids, and weather, all could negatively affect the impact of herbivorous species feeding on weeds. The ability to increase rapidly in numbers is not characteristic of all species of herbivores but is clearly a positive attribute for biological control agents when it results in more intensive effects on host plants.

Sometimes grazing by vertebrates has been used to control weeds, but this is usually non-specific, with vertebrate herbivores feeding on a diversity of plant species and plant parts. Weeds in aquatic ecosystems can completely clog bodies of water and are difficult to control. Herbivorous fish for control of aquatic weeds are the most common type of vertebrate used for biological control of weeds.

Plant pathogens have also been used for controlling specific weed species. In a few cases, host-specific fungi have been used for classical biological control while, in a few more instances, products based on plant pathogens have been registered for inundative release.

Biological control programs have often considered introducing several different agents among the diversity of organisms available, often using several different feeding guilds based on the hypothesis that action of these would be complementary. One individual usually does not kill an entire plant so many individuals of the same species are needed to have an impact. It has been hypothesized that such within-species complementary activity can be extended to encompass different species of natural enemies working together to decimate weeds.

13.2 | Weed characteristics

Just as there is a huge diversity of species of phytophagous natural enemies, weeds vary significantly, ranging from algae to trees. Many weeds are *r*-selected species, characterized by abundant reproduction but poor competitive abilities and these species are excellent at colonizing disturbed habitats. A ubiquitous example of this plant life strategy would be the weeds readily colonizing overgrazed land; these weeds grow quickly when few to no other plants are present as competitors. However, while *r*-selected species have advantages in rapid colonization, this strategy also has weaknesses that can be exploited (see below).

What types of plants are most successfully controlled using natural enemies? Two researchers, Burdon & Marshall (1981), decided to investigate whether plants that reproduce sexually or those that reproduce asexually were controlled more often using classical biological control. Sexually reproducing plants of course grow from seeds, while asexually reproducing plants do not require seeds but grow directly from plant parts. As a result, there is more genetic diversity among populations of sexually reproducing plants than among asexually reproducing plants, many of which grow as genetically identical clones. Analyzing 81 separate control attempts, asexually reproducing plants were controlled more often than sexually reproducing plants. The authors hypothesized this was due to the genetic similarity among plants, which allowed natural enemies to optimize their attacks more easily. In support of these findings, classical biological control programs against the asexually reproducing aquatic weed *Salvinia molesta* (see Chapter 14) have been extremely successful, while control of the

sexually reproducing *Lantana camara* has been very difficult. *L. camara* has pretty flowers and is cloned by gardeners and grown horticulturally in the tropics. There is abundant genetic variability within the species *L. camara* and insects that are effective at damaging one clone can have little effect on others (Crawley, 1989).

Some weeds have proven especially difficult to control worldwide. Do these weeds have attributes in common to help predict the outcome of biological control programs? It seems that plants with better adaptations for withstanding stress can be more difficult to control. Especially among perennial weeds, resistance to control has been associated with a long growing period, large reserves (e.g., thick underground rhizomes or woody stems), good powers of regrowth after defoliation, good ability to replace fruit and seeds after injury, and large seed banks with dormant seeds. If weeds are of poor nutritional quality for herbivores, natural enemies are usually less successful for control. Annual weeds of arable crops are more difficult to control with classical biological controls due to the regular disruption in such cropping ecosystems. All of these characteristics make the stresses that are applied by phytophagous natural enemies less apt to kill weedy plants. However, none of these characteristics, alone or together, has entirely ruled out success of a biological control agent and thus it is once more difficult to predict outcomes of classical biological control programs (Lawton, 1990).

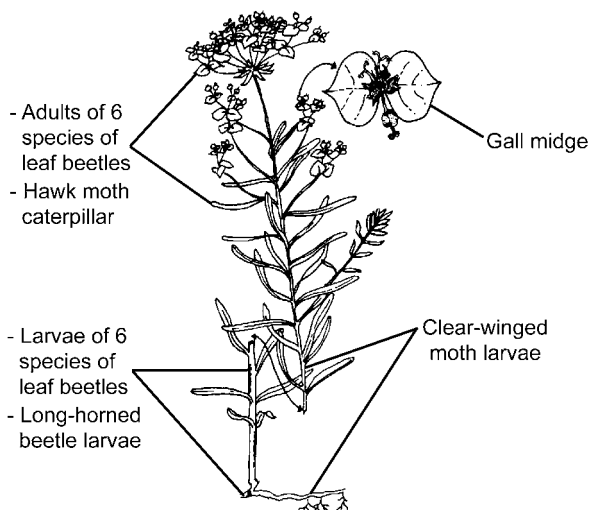
13.3 | Types of injury to plants

While parasitoids and predators used for biological control of invertebrates often kill one or several hosts, individual biological control organisms attacking plants rarely kill them outright (Harris, 1981). In fact, plants usually can survive some degree of attack by herbivores or plant pathogens and still reproduce. Biological control agents are usually most effective in controlling weeds when they increase in numbers and act in concert. Although for weed control we are concerned with weed populations, the effects of natural enemies on weeds of course occur on individual plants. Plants are complex spatially and temporally and different structures that occur at different times can be attacked (Fig. 13.1). There are several major ways that invertebrate natural enemies impact plants by feeding on different parts of a plant.

13.3.1 Reducing flowers and seeds

Attacking flowers and seed-producing structures can reduce the numbers of viable seeds set by plants, although of course this would not impact plants that are already established or plants that predominantly reproduce vegetatively. Therefore, this type of effect would have the greatest impact on annual plants that die each year. Likewise, when seeds are eaten or seed production is disrupted, spread by weed populations is impacted.

Fig. 13.1 The diversity of natural enemies introduced against leafy spurge, *Euphorbia esula*, in North America. Agents released include leaf and flower feeders, stem and root miners and a gall former. (Drawing of leafy spurge courtesy of Lym, 1995.)



For some plant species, destroying reproductive output has proven to be an effective strategy for controlling increase and spread. To control the leguminous tree *Sesbania punicea* in South Africa, a small weevil (*Trichapion lativentre*) from South America that voraciously attacks flower buds was introduced, reducing seed production by 98%. When this was followed by introduction of a seed-loving weevil (*Rhyssomatus marginatus*) that destroyed up to 88% of the remaining seeds, reproduction by this plant was almost entirely halted (Hoffmann & Moran, 1992).

However, in many cases weed densities are not limited by the numbers of seeds; many weeds produce an overabundance of seeds and such species are limited by availability of new locations for seedlings to grow. Based on studies and models of the prolific seed-producer diffuse knapweed (*Centaurea diffusa*), Myers & Risley (2000) suggested that seed feeders are often not likely to be successful agents for reducing existing plant populations.

13.3.2 Direct mortality of plants

Biological control agents rarely directly kill plants, although this can occur if natural enemies increase in numbers and large populations then consume entire plants. Perhaps more commonly, large populations can eat all of the foliage on a plant (defoliation). Many plants such as oak trees can grow a second set of leaves after defoliation, but coniferous trees die if completely defoliated. Even for plants that can refoliate, each time a new set of leaves is produced, the plant progressively uses up storage reserves and this weakens the plant.

Intensive site-specific feeding by herbivores can kill plants if vital functioning of the plant is impaired. For example, mortality of the plant could occur if the water transport system was destroyed. Yet, such directed and effective attacks that quickly kill host plants are not common strategies for the invertebrates that feed on plants. One

could consider that it often behooves phytophagous invertebrates to keep their host plant alive so that they can develop while eating it and so that it is present for their offspring to eat. Therefore, for biological control of weeds, we are looking for the more unusual phytophagous invertebrates that for some reason or in some way over-exploit and kill their host plants, at least when the host plants are at high densities.

13.3.3 Indirect plant mortality

Plants are generally thought to survive with some level of injury and compensate by using storage reserves. Based on this fact, Peter Harris (1986) proposed a “stress hypothesis” for recommending how to impact weeds. He suggested that there is a damage threshold, and that above that threshold, plants cannot compensate for damage and have an increased risk of death. The trick is to exceed that damage threshold for the weed species in question. This can be done indirectly by biological control agents attacking the specific tissues that are most sensitive, such as storage reserves. For example, if carbohydrates are stored in the leaves, then attacking the leaves would be most effective. Also, natural enemies that attack plants during time periods when they are more susceptible can be more effective. When musk thistle (*Carduus nutans*) is growing as a rosette of leaves at ground level it can be killed by defoliation but, once musk thistles are producing fruiting stalks, defoliation is no longer effective for control (Cartwright & Kok, 1990).

Sometimes, for herbivores to “stress” a plant sufficiently to cause mortality, the plant must be growing optimally. One factor that limits growth and development of herbivores can be plant nutritional quality, especially percentage of nitrogen (Mattson, 1980). Although this seems counter-intuitive, there are numerous examples where fertilizer has been added to weeds to improve biological control. While some herbivores do well on poorly growing plants, in some systems herbivores instead grow best on vigorous plants. In these examples, fertilizing weeds made all the difference in strong establishment and activity of phytophagous natural enemies that could provide very effective control when plants were growing optimally. However, when plants were growing without fertilizer, these herbivores were not very effective. In other instances, plants that were stressed by drought were less suitable for herbivore survival and otherwise effective phytophagous invertebrates provided little control when plants were drought-stressed (see Box 13.1). These examples demonstrate that variability exists among those factors driving different weed/natural enemy systems and it is important to understand the relations between the herbivore, the weed, and plant quality to facilitate weed control by herbivores.

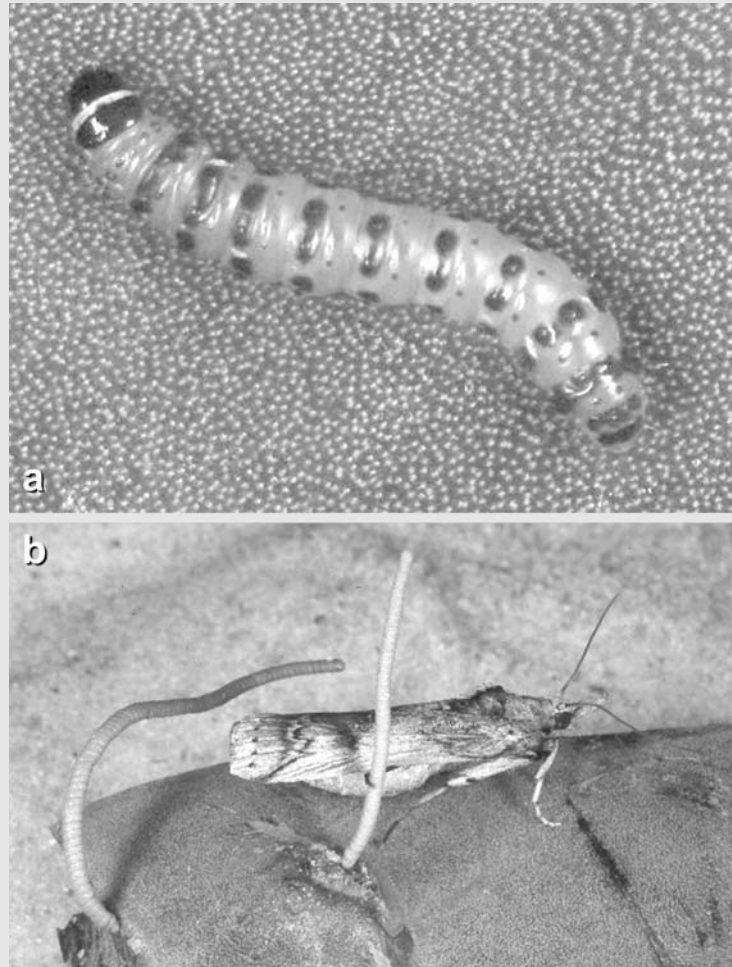
13.3.4 Interactions leading to increased plant stress

Multiple agents

In many programs for biological control of weeds, numerous agents are released against one weed species. Often the released agents attack

Box 13.1 | *Cactoblastis* larvae blast prickly pear cactus

Biological control of prickly pear cactus, *Opuntia* spp., in Australia, demonstrates that different dynamics can occur in different areas due to density-independent factors (White, 1997). Prickly pear was introduced to Australia and infestations increased from 5 million hectares in 1905 to 25 million hectares in 1925, by which time this cactus occupied most of the habitats that would support its growth, creating impenetrable areas that previously had been used as rangeland. In 1926, the moth *Cactoblastis cactorum* from Argentina was released. These caterpillars, aided by microbial rots, began destroying prickly pears. By 1934, most, but not all,



a. Caterpillar of the pyralid *Cactoblastis cactorum* (length 25–30 mm), which attacks prickly pear cactus is bright orangish-red with black transverse stripes. b. Adult moth (wing span 2–3.5 cm) and eggs that are laid on top of each other to form long sticks resembling cactus spines. (Photos by J. Carpenter, USDA, ARS.)

plants had been destroyed. With no food, huge numbers of caterpillars starved to death. There was one more cycle during which the remaining plants began to regrow but then *C. cactorum* found them and increased again to finally stabilize at a low density of prickly pear by 1939. The infestation of prickly pear had been reduced by over 99% in Queensland, where the moth and prickly pear populations then persisted in an equilibrium of sorts. The equilibrium densities of the cactus and moth now vary by weather, climate, and region.

In southern Australia, *C. cactorum* populations are limited by cooler temperatures and prickly pear thus persists at higher densities than in Queensland to the north. In drier western parts of the prickly pear range during droughts the prickly pears may die back. In more favorable coastal areas, even during a drought prickly pear continues to grow, although the water-stressed plants are less suitable for survival of the *C. cactorum* caterpillars. Effects of poor plant quality are exacerbated by occurrence of high temperatures leading to caterpillar population decline. Thus, prickly pear densities increase while *C. cactorum* populations decrease until wet conditions return.

A similar situation is seen in areas with soil types that provide poor nutrition for prickly pear. When growing under these conditions, the plants produce highly mucilaginous sap in which *C. cactorum* larvae drown. If nitrogen fertilizer is applied so that plants are healthy and grow normally, the plants once more become susceptible to *C. cactorum*. Thus, in these regions, the weather and soil, factors not dependent on plant density, can strongly affect the activity of the natural enemy.

the plant in different ways (seed feeders, leaf feeders, stem feeders, root feeders) or during different seasons, thereby increasing the overall plant stress. Examples have shown that this strategy can be very successful toward hastening the demise of weeds.

In some instances, a released herbivore can create an injury to a plant allowing entry by naturally occurring plant pathogens, leading to weed mortality. One famous example is the caterpillar *Cactoblastis cactorum*, attacking prickly pear cactus that had been introduced to Australia (Box 13.1). The caterpillars feed gregariously within the fleshy cactus pads, but do not eat the entire pad, and then drop to the ground to pupate in the leaf litter. After the caterpillar creates wounds, native opportunistic microbes move into this nutrient-rich wound environment and go on to kill the cactus pad (White, 1997).

Abiotic stress

Plants store carbohydrates to use as reserves when they need energy. As a part of the stress hypothesis, if plants are defoliated, they will use storage reserves for regrowth. If they subsequently are also stressed by drought or winter cold, this can result in mortality because reserves are gone. Cinnabar moth caterpillars (*Tyria jacobaeae*) reduce root reserves when eating tansy ragwort (*Senecio jacobaea*) and this can lead to increased plant mortality when frost occurs (Harris *et al.*, 1978).

Competing plants

Biological control agents are also aided if highly competitive native plants occur along with the weeds. All plants compete for light, water, nutrients, and space. If natural enemies stress weeds, the weeds will not be as effective at competing for these resources against good competitors. When the weedy fiddleneck (*Amsinckia intermedia*) is attacked by a flower gall nematode (*Anguina amsinckiae*), wheat becomes the more successful competitor of these two plants and outcompetes this weed.

13.4 Regulation of weed density by herbivores

To investigate the ecological theory behind how herbivores and pathogens control weeds, we turn to the study of plant ecology. However, in this case, we are specifically not investigating the normal flora, native plants in undisturbed natural ecosystems, but instead we are interested in plants whose populations are “out of control,” often weeds that have been introduced. The discussion of population regulation theory based on predators or parasites and prey or hosts (Chapter 6) is directly applicable to weeds and herbivores. The same issues exist over the relative importance of density-dependent relations between natural enemies and pests, stability of the system, importance of metapopulations for recolonization, and importance of refuges in sheltering portions of populations. Below, we will explore some relevant issues more specific to the basis for regulation of weeds by phytophagous natural enemies.

13.4.1 How do herbivores regulate plant populations?

Every plant species, even those most resistant to herbivory, is associated with organisms that have evolved to eat it. Our question then is why the terrestrial world is green if there are so many herbivores. Herbivores consume an average of only 10–20% of the annual net primary production in terrestrial (“green”) ecosystems (Hartley & Jones, 1997). In contrast, the aquatic world could be green but is not. It has been estimated that 80% of the available primary production under water is removed by aquatic herbivores. Understanding why terrestrial plants remain largely uneaten despite the diversity of herbivores and plant pathogens that occur is a fundamental question in plant ecology. For practical reasons, we are often interested in keeping areas green, that is, preventing herbivory to protect crop plants and forests. Relative to biological control of weeds our interest is the opposite: what is necessary for herbivores and plant pathogens to be successful in controlling weedy vegetation?

There have been numerous theories proposed to explain why the terrestrial world is green. One idea is that predators, parasites, and pathogens attacking herbivores keep herbivores rare. Another view is that herbivores are rare or ineffective at controlling plants because they exist “between the devil and the deep blue sea” (Lawton &

McNeill, 1979). Their populations are controlled in part by their natural enemies (the devils) but also by the fact that plants are often not very good food (the deep blue sea). Many plants do not supply the abundant and diverse nutrients needed by herbivores, and the supplies of nutrients for herbivores can vary drastically in time and space. Over evolutionary time, plants have also countered attacks by herbivores and pathogens through evolved defenses. Defenses take many forms based on the type of herbivore exerting selective pressure. For example, thorns evolved to prevent feeding by vertebrate herbivores, and many plants have indigestible or fibrous parts that also help to protect them. Some plants can simply tolerate loss of parts without affecting their ability to produce offspring successfully. Many scientists have been fascinated by the chemicals in plants that are repellent or deterrent, or inhibit digestion. These can be primary plant metabolites that can have negative impacts when either too much or too little of compounds such as proteins, carbohydrates, and vitamins are present (Berenbaum, 1995). Some plants also produce specialized secondary metabolites that can provide excellent protection. Examples of such secondary plant chemicals are alkaloids, cyanogens, and terpenoids. The furanocoumarins found in parsnip or the cardiac glycosides found in milkweed (*Asclepias* spp.) make these plant species unsuitable for many herbivores. However, a few herbivores have evolved the ability to feed on these chemically defended plants, despite the presence of the nasty secondary plant compounds, and these herbivores then encounter less competition for their source of food.

As with herbivore populations, plants remain at any given population density due to interactions between those factors killing them and those factors affecting their growth and reproduction. These are now frequently referred to as the “top-down” and “bottom-up” forces, respectively, that regulate plant populations. For example, with “top-down” regulation, the activities of herbivores could be key in determining densities of plants, while with “bottom-up” regulation, population densities could be affected most profoundly by the availability of space, nutrients, water, and light. This way of investigating regulation of plant populations can be useful for looking at conditions and interactions among herbivores and pathogens that at times effectively control weeds. In fact, there is agreement that in natural systems, plant populations are often controlled by some combination of bottom-up and top-down forces. But, we’ve agreed that weeds are generally not in synchrony with the natural ecosystem in which they occur. Therefore, for biological control of weeds, we are trying to establish situations where top-down forces are successful in reducing and then regulating weed populations.

For herbivores to be effective in controlling plant density, their populations must be able to respond to weed populations by being free from their own regulators. With weedy plants, there is often no lack of food for the herbivore, and thus bottom-up forces are not limiting populations of the herbivore (although herbivores can be influenced by plant quality so presence of a plant species does not

always translate to optimal food). When exotic herbivores are released in new areas, the herbivore often escapes from the top-down forces with which it coevolved (predators and parasites) and that regulate its populations in its area of endemism. Thus, the herbivore can increase in response to abundant host plant populations and its effect on the weed will largely be determined by the inherent ability of that herbivore to increase.

13.4.2 Weed population ecology

After studying insect and mite pests, there is the tendency to think of weeds based on individual plants. However, it is the plant biomass not the individual plant that is important to reduce with weed control programs. Plants of the same species can vary dramatically in size and can be composed of many different parts that change with time. Many biological control agents attack only certain parts of plants, so thinking of plants as being composed of modules can be helpful.

The population levels of plants can also be thought of as being determined by a balance between activation and inhibition (McEvoy *et al.*, 1993). An example of this “activation-inhibition” hypothesis could be the balance between the occurrences of weed outbreaks caused by local disturbances that provide new, open habitats, such as humans building a road, with subsequent weed colonization (activation) versus insect herbivory or plant competition reducing weed populations (inhibition). Of course, the goal with biological control is to shift this balance toward inhibition of weed populations.

How do we approach increasing weed inhibition, increasing the “top-down” forces, for weed control? The best known general tactic is to work toward decreasing the growth rate of the target organisms, the weeds. As an example, we will use the weed tansy ragwort, a poisonous pasture weed endemic to Europe and now introduced to five continents and several islands (Schroeder, 1983). Tansy ragwort grows best if competition from other plants is reduced or absent and herbivores are absent. Tansy ragwort is typical of the so-called *r*-selected, ruderal or “fugitive” species that are good colonizers and fast growers but cannot compete well with other plants. In northern California, the cinnabar moth was introduced from France in 1959 for control of tansy ragwort (Fig. 13.2). While caterpillars of this species caused some defoliation, only partial control was achieved. In 1969, the leaf-feeding beetle *Longitarsus jacobaeae* was introduced from Italy to California, Oregon, and Washington in another attempt at control (Fig. 13.3). At sites in California and Oregon, this leaf beetle was extremely effective. Adults of *L. jacobaeae* feed on foliage and larvae feed on the roots. The root-feeding larvae stress tansy ragwort plants during winter and spring and, with sufficient defoliation, the plants are severely weakened. With ragwort under stress, the other plants in the area could outcompete ragwort and populations of this poisonous weed were reduced. After both of these phytophagous insects were established, Oregon livestock losses due to tansy ragwort



Fig. 13.2 Larva of the cinnabar moth, *Tyria jacobaeae* (25 mm long) released for control of tansy ragwort. (Photo by Noah Poritz.)



Fig. 13.3 The leaf beetle *Longitarsus jacobaeae* (adults 2–4 mm long) released for control of tansy ragwort. (Photo by Noah Poritz.)

poisonings were reduced by 99%, saving producers more than US\$5 million annually (Julien & Griffiths, 1998).

In keeping with Harris' stress theory (1981), weed inhibition can be aided by increasing the overall stress impacting weeds so that the damage threshold is exceeded. The action of plant-feeding individuals places a stress on plants but sometimes unless other stresses also occur, plants do not die. Such supplementary stresses can include climatic conditions, as found with the cinnabar moth attacking tansy

ragwort in Canada. When this weed was growing in British Columbia, every summer the caterpillars of the cinnabar moth ate all of the leaves from the plants but, after they finished feeding and pupated, plants still had enough storage reserves to rally and produce more leaves. In comparison, on well-drained sites in the colder Atlantic Canada area where cinnabar moth was released, plants were also defoliated. However, with the short growing season in this colder climate, the plants did not have enough time to regenerate leaves before colder weather set in. But they tried. The plants would be actively producing another set of leaves when cold weather began and, in this condition they were more sensitive to cold and then died from the low winter temperatures. As a result, tansy ragwort virtually disappeared from cinnabar moth release sites in Atlantic Canada but was not as well controlled in British Columbia (Harris *et al.*, 1978).

Due to the inherent variability in biological systems, not all types of plant feeders are found attacking each plant species. However, in most cases, there are numerous species of natural enemies that can be introduced for control of a weed species and the choice of which ones to pursue and introduce is not a trivial decision. Ecological information can help identify the “Achilles heel” in a weed life cycle in order to target the more sensitive growth stages or parts of the plant. However, it is important to release control agents that are not themselves attacked by local predators, parasitoids, and pathogens so that their populations can build and become as abundant as possible. For example, when the cinnabar moth was released in southern Australia to control tansy ragwort it did not become established due to parasites, diseases, and native predators, especially a species of predatory scorpionfly (*Harpobittacus nigriceps*).

A successful approach has been to study plant populations and understand which transitions among life stages are most sensitive to herbivory (McEvoy & Coombs, 1999). Tansy ragwort is a long-lived plant and it seemed to Peter McEvoy that the transition from one-year-old plants to two-year-old plants is a sensitive stage when plants undergo the transition from being non-reproductive to being reproductive. This information added to the natural enemy species available pointed toward introducing a leaf beetle that stresses plant growth in juvenile stages instead of a flower-feeding moth. While the flower feeder can remove many seeds, seeds are rarely a limiting part of the tansy ragwort life cycle so this natural enemy was considered largely unnecessary. In addition, by understanding the ecology of this system, other limiting factors were identified that could then be used in tandem with releases of herbivores. Researchers suggested that tansy ragwort is a poor competitor but a good colonizer so plant competition should be increased, as well as releasing herbivores, by identifying areas slated for control efforts and seeding them with non-weedy plants that are strong competitors. In addition, disturbance frequency and intensity in the weedy areas should be decreased so that new spaces for tansy ragwort to colonize were not created.

If multiple natural enemies are introduced, there is the potential for competition among herbivores both on the same plants and across plant populations. However, the most important factor in determining what types of natural enemies and how many species to introduce is what is necessary to increase overall damage to the weed. A study by Myers and Harris (1980) investigated two small, patterned-winged fruit flies (Tephritidae: *Urophora quadrifasciata* and *U. affinis*) attacking weedy knapweeds (*Centaurea diffusa* and *C. maculosa*). Feeding by larvae of these flies causes galls to form in the seed heads, thereby greatly reducing seed production. When both species occurred together there was greater destruction of seeds than when either species occurred alone. While these flies coexisted and had a greater effect together than alone, overall they did not control this weed. Models have suggested that better agents would be herbivores that feed on the basal rosettes of knapweeds, the persistent structure that must be killed so that plants do not grow back. However, practitioners in the biological control of weeds must always work within the bounds of the diversity of herbivores that have evolved to feed on that plant species and that can be manipulated; so, the ideal herbivore that a practitioner of biological control might have in mind does not always exist or perhaps is too difficult to rear and release. In addition, the host specificity of agents potentially being considered for release is a critical factor regarding whether a species can be used (see Chapter 18).

13.4.3 Weed populations through time

Weeds differ from insect pests because they occupy fixed locations for much longer than mobile insect or mite prey. In some ways, this situation is more similar to very sessile invertebrates such as scale insects or female whiteflies. The importance of one of the attributes considered critical for invertebrate predators and parasitoids, excellent searching ability, is less important with herbivores because weeds are relatively easier to locate; weeds are essentially sessile when compared with mobile pestiferous invertebrates. Interestingly, biological control programs against sessile invertebrates have been more successful, as has biological control of weeds (see Chapter 3). Of course, over longer periods of time, weeds do move when seeds are dispersed. *r*-selected weed species often produce abundant seeds and thus readily relocate and begin growth in newly disturbed areas. Thus, there is generally a lag time before herbivores find newly established populations of weeds that have created new metapopulations. An example of this is provided by a leaf-feeding beetle established for control of Klamath weed. After establishing and controlling this weed, populations of this beetle persist by feeding on the few Klamath weed plants growing in the shade. It is only when a new Klamath weed population becomes established in a sunny area that the beetle population increases to control the weed when it is growing in the sun (Box 13.2).

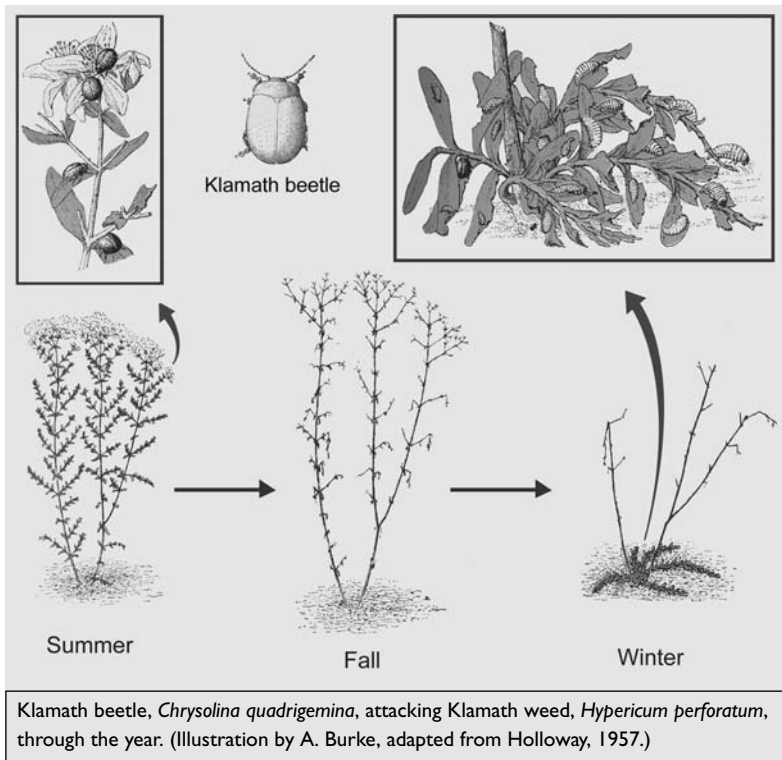
Box 13.2 | Klamath weed in the sun and shade

Hypericum perforatum is known as St. John's wort in England, where it is native. Some time after it was first found in the Klamath River area of northern California in about 1900, this weed earned its second name, Klamath weed. These plants are somewhat innocuous in appearance, being erect, multi-stemmed perennials that are 30–100 cm tall with small yellow flowers. However, they produce a compound, hypericin, that is toxic to livestock. *H. perforatum* has tiny seeds that readily stick to the oily hair and hide of animals and its spread was associated with movement of sheep flocks as well as with being transported with hay and seeds of other plants. Klamath weed therefore increased and by 1944 it covered two million hectares of rangeland in the northwestern USA and Canada. It outcompeted good forage and cattle and sheep lost weight when eating it. Chemical methods to control this weed were unsuccessful and land values plummeted. Interestingly, as early as 1922, Harry Smith, an early leader of biological control in California, suggested introducing plant-feeding insects to control Klamath weed, but he was ahead of his time and his suggestions were not seriously considered.

This same weed had also made its way to Australia and entomologists there had been battling it by introducing *Hypericum*-feeding insects since 1929. Dr. Smith had been closely following the progress in Australia and by 1944, he received permission to import three European species of beetles that showed promise in Australia. World War II was raging in Europe so these European beetles were obtained from Australia instead of Europe. However, this posed a bit of a snag because the seasonal timing of the beetles was 6 months off. Two species of leaf-feeding beetles of the genus *Chrysolina* arrived in a summer resting state but, when misted, they became active again and began laying eggs within a few weeks. Host range testing determined that both beetles would feed only on Klamath weed.

These beetles were released in 1945 and 1946 and it soon became evident that *C. quadrigemina* was the dominant of the two species of leaf beetles, having better synchrony with the climate and growth phases of the plant in release areas. These beetles have a one-year life cycle, with all adults feeding on foliage during spring and then remaining inactive all summer. Eggs are laid in late fall and both larvae and adults eat the winter basal growth of the plants, killing some plants. The destruction of Klamath weed by *C. quadrigemina* occurred year-round so the plant had no opportunity to recover. Directly after release, the beetles overexploited the plants and some Klamath weeds were killed directly by their feeding. For other plants, after about three years of damage, the root reserves were completely utilized and the weeds, in competition with surrounding plants, died. The beetles had plenty to eat and established well. From one colony of 5,000 beetles that had been released, more than 3 million beetles were collected for redistribution throughout the Klamath weed range.

The activity of these beetles in California far surpassed what had been seen after releases in Australia. Within 10 years, Klamath weed was an uncommon roadside weed in California, with its abundance reduced by 99%. As a result, land values increased by three to four times and cattle and sheep gained weight. It was estimated that these beetles resulted in savings of over \$20 million between 1953 and 1959 and savings continue today.



Today, Klamath weed can still be found but only in shady areas. While *C. quadrigemina* and a beetle with root-boring larvae, *Agrilus hyperici*, that was also introduced can be found, they do not kill all plants growing in the shade. The extent to which the leaf-feeder and root borer interfere with each other has been discussed but the fact remains that neither species, alone or together, is as efficient at killing Klamath weeds in the shade as *C. quadrigemina* is in the sun (Huffaker *et al.*, 1971). If Klamath weed populations increase and spread from the shade into sunny areas, *C. quadrigemina* quickly responds and kills these plants. In the majority of locations where *C. quadrigemina* has been successful, the normal rangeland plants have re-established and the history of high populations of Klamath weed in those areas is no longer apparent and has largely been forgotten.

13.5 | Measuring impact of biological control

Evaluating the results of biological control projects is seldom straightforward but the challenges presented by weed control differ in some ways from those encountered with biological control of invertebrates. Usually, densities of plants in the native flora have not been measured prior to invasion of a weed. Although we often have no quantification of the plant density pre-invasion, it is still important to document the densities of weeds at the time of release as well as the effects of biological control programs after releases. Unfortunately, the effects

of natural enemies on weed populations have often not been quantified and thus the impact of biological control programs on weeds is often judged only subjectively. At the least, the initially infested area should be estimated with subsequent quantification of decreases in areas covered by weed species. Of course, over time, the level of control can change dramatically as weed populations decrease, as with the caterpillars of *Cactoblastis cactorum* feeding on prickly pear cactus in Australia. Prickly pear cactus in an area can all be killed by *C. cactorum* so that the *C. cactorum* population becomes locally extinct. Prickly pears can then be reintroduced, often when pieces of prickly pear are carried by floods or seeds are carried by birds or other animals. Cactus populations then increase until *C. cactorum* disperses to locate this new patch and then controls this newly established prickly pear population (White, 1997). Therefore, monitoring weed populations is never really finished, because weeds, once gone from an area, have great potential to reinvade.

FURTHER READING

- Bellows, T. S. & Headrick, D. H. Arthropods and vertebrates in biological control of plants. In *Handbook of Biological Control*, ed. T. S. Bellows & T. W. Fisher, pp. 505–516. San Diego, CA: Academic Press, 1999.
- Harris, P. Stress as a strategy in the biological control of weeds. In *Biological Control in Plant Production*, ed. G. C. Papavizas, pp. 333–340. Totowa, NJ: Allanheld, 1981.
- McEvoy, P. B. & Coombs, E. M. Biological control of plant invaders: Regional patterns, field experiments, and structured population models. *Ecological Applications*, 9 (1999), 387–401.
- Myers, J. & Bazely, D. *Ecology and Control of Introduced Plants*. Cambridge: Cambridge University Press, 2003.
- Rosenthal, G. A. & Berenbaum, M. R. (ed.). *Herbivores, Their Interaction with Secondary Plant Metabolites*. San Diego, CA: Academic Press, 1991.
- Stamp, N. E. & Casey, T. M. (ed.). *Caterpillars: Ecological and Evolutionary Constraints on Foraging*. New York: Chapman & Hall, 1993.

Phytophagous invertebrates and vertebrates

Among projects for the biological control of weeds, the vast majority would be classified as classical biological control and the majority of programs have used phytophagous arthropods.

14.1 Invertebrates

In nature, there are many invertebrate species feeding on any plant species and for biological control purposes, decisions must be made as to which species to concentrate efforts on. The invertebrates released to control weeds are diverse, but insects have predominantly been used along with a few mites and nematodes (Table 14.1). In a few instances, snails, crayfish and tadpole shrimp have been tried against aquatic weeds. With such diversity in the invertebrates used, commonalities in their biologies are few. However, we can summarize by saying that different invertebrates can be found utilizing virtually all of the different parts of plants.

The majority of insects that are used for biological control bite off pieces of plants and chew them, as seen with weevils, leaf beetles, and caterpillars. Although the amount that each individual eats is quite small, populations of these species can increase to large numbers when weeds are abundant and control is then achieved by the synchrony of action of many individuals within the population. Many of the most successful natural enemies are holometabolous, having immature stages capable of limited dispersal but metamorphosing into more mobile adults that move to new locations to lay eggs. In fact, holometabolous species comprise 86% of the successful invertebrates released. For these groups, the immature stages can be extremely specialized in the areas they inhabit because they can later change to completely altered, highly mobile adults that will lay eggs in a new location. This is especially advantageous if, while these individuals were developing, they killed the host plant on which they occurred and would then starve if they could not leave. While some phytophagous insects used for biological control live externally on the foliage, larvae of others are often legless and live within protected locations in the

Table 14.1 The diversity of invertebrates released for classical biological control of weeds

Group	No. species	Establishment (% of species)	Successes (% of species)
<i>Insecta (Insects)</i>			
Beetles (Coleoptera)	109	66 (61%)	33 (30%)
Caterpillars (Lepidoptera)	82	46 (56%)	15 (18%)
Bugs and scales (Hemiptera)	19	15 (79%)	8 (42%)
Flies (Diptera)	35	25 (71%)	4 (11%)
Thrips (Thysanoptera)	4	2 (50%)	1 (25%)
Sawflies, galling and seed-feeding wasps (Hymenoptera)	4	3 (75%)	2 (50%)
Grasshoppers (Orthoptera)	1	1 (100%)	0 (0%)
Mites (Acarina)	5	3 (60%)	2 (40%)
Nematodes	1	1 (100%)	0 (0%)

Summarized from Bellows & Headrick, 1999; data from Julien, 1992.

plant tissues, for example boring into stems or roots, and then dispersing as adults. Among those listed in Table 14.1, the groups most often used include beetles, caterpillars, scale insects (within Hemiptera), and flies. The basic biologies of some of the most commonly used groups are reviewed below.

14.1.1 Leaf beetles (Order Coleoptera: Family Chrysomelidae)

Leaf beetle adults are generally less than 15 mm in length with bodies longer than wide and they are usually rather robust. All beetles are holometabolous so larval leaf beetles are very different in appearance from the adults. For some species the elongate larvae feed externally on the foliage and are able to hold on with their short legs. For other groups within this family, larval stages tunnel in stems and roots and these larvae usually have less morphological differentiation and reduced legs. Leaf beetles have chewing mouthparts as both adults and larvae and both stages are phytophagous, although adults sometimes eat flowers as well as foliage. Therefore, although they are holometabolous and could specialize on very different types of food, adults and larval leaf beetles often feed on the same food. However, for some species, larvae and adults use different parts of the same plant. Having both adults and larvae feeding on the same plant could help to explain the effectiveness of these beetles for biological control. The flip side is that some species of this family of effective plant-feeding beetles can be serious pests of crops.

14.1.2 Weevils (Order Coleoptera: Family Curculionidae)

Weevils are often less than 15–20 mm in length with the head narrowed into a protuberance called a rostrum that is characteristic of this family. For some species the rostrum is quite short and blunt (see Box 18.2) while for others it is long and narrow (see Box 14.1). The

antennae are attached about half way down the rostrum. Due to the appearance of the rostrum, these beetles are sometimes called snout beetles. Weevils are usually dark or cryptically colored so that it is difficult to see them in nature. If disturbed, they frequently draw in their antennae and legs and fall to the ground remaining motionless and are then very difficult to see.

For plant-feeding weevils, both larvae and adults are phytophagous. The chewing mouthparts of the adult are somewhat hidden at the end of the rostrum and are often used to drill holes in fruits, nuts, and other plant parts. Larvae are C-shaped and whitish and almost always without legs, living in protected locations, chosen when adult females lay eggs. Larvae often bore in roots and stems or fruiting structures.

14.1.3 Pyralid caterpillars (Order Lepidoptera: Family Pyralidae)

Pyralids are small and rather delicate moths whose front wings are elongate or triangular. This is a large and ubiquitous family with species occupying both terrestrial and aquatic habitats. For many species, the larvae are smallish and usually live in shelters, either by webbing together leaves or shoots or by living within shoots, stems, or seed heads. For some species, larvae are gregarious, living in groups. Larvae have chewing mouthparts although adults have drinking tubes, as do butterflies, and only imbibe water and nectar.

This family of moths is included among the most-used agents for biological control of weeds in part because it includes *Cactoblastis cactorum*, which has been introduced successfully in many places. This is the moth that has been used extensively against prickly pear cactus in many different countries (Box 13.1).

14.1.4 Scale insects (Order Hemiptera: Suborder Homoptera: Family Dactylopiidae)

Members of the Sternorrhyncha include scale insects, aphids, mealybugs, and whiteflies. These small insects all have a very specialized feeding strategy, using piercing-sucking mouthparts that they insert into plants to drink living plant sap. Because the plant sap is low in essential nutrients, large quantities are imbibed and, after nutrients are removed, the excess liquid is excreted. This excreted sugar solution, “honeydew,” can frequently be found on plants near colonies of these insects. Ants that “tend” aphids and scale insects feed on this copiously produced sugar solution and, in return, defend these herbivores.

Scale insects in the family Dactylopiidae have been used extensively for biological control of weeds. The life cycles of scales are exceedingly complex. Eggs are laid under the female covering or “shell” and hatch to become mobile crawlers. The crawlers disperse and then settle down and begin feeding as nymphs, forming a shell to protect themselves. Females are hemimetabolous, having few morphological features and gradually maturing within a shell. Males are more holometabolous with similar development through the settled

nymph but after this, males pupate and then emerge as adults with wings. To many human observers, a colony of scale insects would look like only numerous small bumps on some part of a plant. As with the pyralids, the reason this group is among the most used is that species of *Dactylopius* have been used extensively for control of prickly pear cactus. Members of this scale genus are often called cochineal and all produce red pigment, historically leading to mass production of *D. coccus* as a source of carmine dye.

14.1.5 Less frequently used groups

In addition, wood- and stem-boring beetles have occasionally been released as biological control agents as well as some types of true bugs that feed on seeds or plant fluids within leaves, petioles, and stems. Flies that have been used either live in mines in the small area between the top and bottom surfaces of leaves or form galls that disfigure plants while preventing abundant plant growth and reproduction. Some small wasps that have been used cause formation of galls and wasp relatives, the caterpillar-like sawflies, have also been used to control weeds.

14.2 Successful attributes of invertebrate herbivores

There are two characteristics particularly important when choosing insects to release for biological control of weeds: host specificity and the ability of the natural enemies to respond to weed populations in a density-dependent manner. Classical biological control is the principal strategy used against weeds and for this strategy to be successful, it is often thought that natural enemies must increase in numbers when there are more weeds and decrease once weeds are rare (density dependence). It is considered optimal that after natural enemies decimate weed populations, they persist in the environment when weed populations are low. Therefore, if weed populations increase again, as, for example after a disturbance, control agents are present and can respond by multiplying rapidly, resulting in subsequent suppression of the weed. Alternatively, if natural enemies do not persist but disperse readily or regularly, they can reinvade the area in case of rebound or reintroduction of the weed. It is also optimal for phytophagous insects to be able to tolerate high densities in order to be able to damage a host plant severely. For example, a leaf-mining buprestid beetle *Taphrocerus schaefferi* was considered a poor choice for control of yellow nutsedge (*Cyperus esculentus*) because larvae are cannibalistic within leaves, so population increase would of course be limited. In addition, larvae of this miner were plagued by parasitoids that limited the ability of this species to increase in density and larvae principally cause damage when leaves are senescing anyway.

Phytophagous species must be able to find new patches of weeds as the weeds initially spread after introduction, so they can increase

in response to increasing weed density. How do phytophagous insects find their host plants, especially when the plants are at low densities? Usually, this choice is made by the ovipositing adult, which is important for holometabous insects because immatures often have limited movement. Clearly, winged adult moths or flies can travel much further than their immature stages, caterpillars and maggots, respectively, so it makes sense that the adults find host plants.

Over a long distance, dispersing phytophagous insects are known to utilize shapes and silhouettes, such as trees on the horizon, for orientation. Recent research on shorter distance location of crucifers by phytophagous insects (Finch & Collier, 2000) suggests that host plant selection by adults can be divided into three stages. First, host plant odors indicate to a dispersing insect that it is flying over appropriate host plants and landing is stimulated. Second, phytophagous insects use visual cues to specifically land on green surfaces and not on the soil. Third, using the chemical receptors on its legs and mouthparts, the adult samples the plant on which it has landed. The insect will lay eggs only after it has received sufficient stimuli; several landings on host plants can be required for reception of sufficient stimuli before eggs are laid.

Fascinating studies of chemical ecology have investigated the use of specialized plant chemicals by phytophagous natural enemies. It has been assumed that insect species with narrow host plant ranges, such as those that would be preferred for use in biological control, often rely on specific chemical cues for finding their host plants. Plant species produce a great diversity of different chemicals and many of these chemicals, called secondary plant compounds, are not required for growth and survival of the plant but are thought to have evolved for plant protection. Individual phytophagous species responded to these compounds through time by evolving defenses to overcome the noxious chemicals of certain plant species. In doing so, there is probably a cost because the phytophage must then detoxify or sequester these chemicals. However, adapting to a chemically defended species of plant has advantages because the phytophagous species then has fewer competitors utilizing the same food source. Another advantage for insects using these chemically defended plants is that the plant chemicals are species specific and can be used for accurate host plant location. Cruciferous plants such as cabbage, mustard, and broccoli all have glucosides that protect them against many insects but are used by specialized herbivores as phagostimulants (substances stimulating feeding) or as stimulants for oviposition.

Host plant choice is actually influenced by many factors that have different priorities and perhaps no general rules are applicable for all species. Plants are not found or chosen by phytophagous insects only on the basis of plant chemistry or on the fact that they are green. Plant morphology and seasonal development can influence plant choice if the phytophagous invertebrates require only a specific stage in the plant life cycle such as the seeds or flowers. Environmental factors can influence whether a plant is chosen. Plants growing in

sun or shade may be preferred, just as stressed, non-stressed, or vigorously growing plants may be preferred. Plants growing in dense populations can attract more herbivores. While in an area packed with acceptable host plants, these phytophagous insects may also feed to some extent on less-preferred plants that unluckily happen to be in the same vicinity. Readiness of adults to oviposit can also influence plant choice; when a female holds many eggs that are mature and ready for oviposition, she may be less selective regarding which host plant to use for oviposition.

14.2.1 Host specificity and safety testing

As with parasitoids and predators, some herbivores feed on only one or a few host plant species while others feed on numerous species. For biological control of weeds, understanding the host specificity of candidate organisms for release is of prime importance. Researchers must confirm that the phytophagous natural enemies being considered for release will not significantly impact the native flora, especially if rare, threatened, or endangered plant species are of concern. Of course, natural enemies are also always tested to make certain that they would not impact plants that provide food or fiber for humans. These issues will be discussed in more depth in Chapter 18 but, because host specificity is so important to biological control of weeds, here we will briefly discuss issues specific to phytophagous insects.

Methods based on natural enemy biology have been developed for testing the host specificity for herbivorous arthropods. Both acceptance of plants by adults for oviposition and suitability for development of immatures must be tested. Different stages of insects can react differently to plants and they do not always react the same way. Adults are known to at times lay eggs on plants that are not optimal for development of immatures. Conversely, plants on which immatures can develop are not always accepted by adults for oviposition. The bottom line is that the host range is usually determined by the stage of the phytophage that is most selective. For example, adults of fruit flies (Tephritidae), longhorned beetles (Cerambycidae) and weevils (Curculionidae), show narrower host acceptance than their larvae, while for many moths and butterflies (Lepidoptera), the opposite is true and the caterpillar is more selective than the egg-laying adult female moth or butterfly. Determining host specificity using the developmental stage with the narrowest host acceptance is therefore the safest approach. Methods used for testing different stages of a candidate biological control agent as well as for choosing which plant species to test are discussed further in Chapter 18.

14.3 Strategies for use of phytophagous invertebrates

Classical biological control has been the principal strategy used for biological control of weeds with invertebrates.

14.3.1 Classical biological control

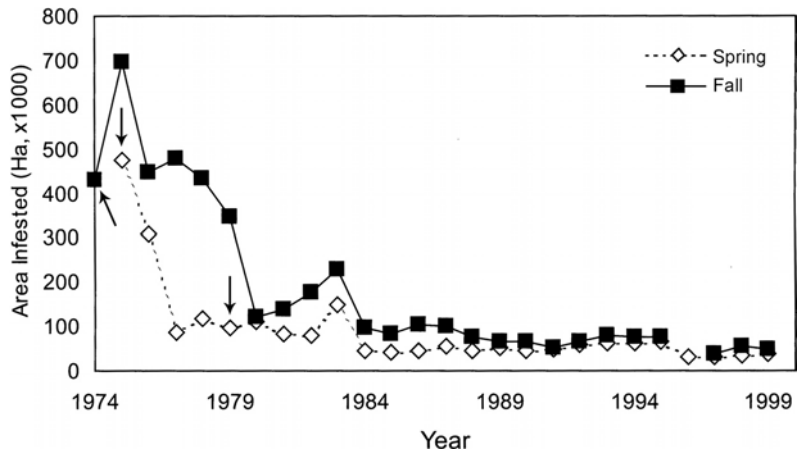
Some of the world's worst weeds have been successfully controlled using classical biological control, but generalizations about what makes for a success are difficult due to the diversity of types of programs and the variety of ways in which results have been evaluated. There is no one correct way to evaluate classical biological control of weeds and many authors have evaluated programs from different angles or not at all. Understandably, complex and often subjective results from classical biological control releases are difficult to summarize under the characteristic categories of success (see Chapter 3). The subjectivity in classification of results, for example establishment, complete control, partial control, which come from many different sources and utilize different data for evaluation, leaves an unknown level of precision. In fact, reports of results are only as good as the data used for evaluation. Unfortunately, long-term collection of quantitative data after releasing natural enemies has been conducted too infrequently. Also, overall summarizations can be skewed by weeds for which control has been attempted with the same agents in country after country. Two examples are the many successful efforts to control prickly pear species, *Opuntia* spp., before 1950 in many countries and the much less successful assaults on the more recalcitrant *Lantana camara* in numerous countries between 1950 and 1970. For biological control of weeds, to analyze success properly, a number of years should be allowed after release and before evaluation of establishment of a natural enemy. Another period of time (up to 10 years after establishment) should be allowed for a natural enemy to have a chance to increase and demonstrate its contribution to control, before a program is evaluated. As can be seen by the example of waterhyacinth in Louisiana, two weevils and a pyralid were introduced over a span of 5 years. Although decreases in the area infested were precipitous in the first few years, this system required a total of 10 years before control seemed to have stabilized and evaluation could be realistic (Fig. 14.1).

Programs for the classical biological control of weeds can be evaluated in numerous ways. We will look at the success by weed species, use of different types of invertebrates and use in different areas of the world.

Weed species

In a 1998 summary of introductions, Julien & Griffiths reported that 133 weed species from 40 plant families had been the targets of classical biological control projects. The majority of these projects had been aimed at perennial, terrestrial weeds. Weeds in the Asteraceae and Cactaceae were most frequently being addressed. There have also been some notable successes against aquatic weeds. However, there are numerous weeds that have not been targeted by programs for the biological control of weeds. A list created in 1977 named 18 species as the world's worst weeds and 10 of these were grasses (Holm *et al.*, 1977). However, historically, weedy grasses were not targets for

Fig. 14.1 Reductions in area of Louisiana infested by the aquatic weed waterhyacinth, *Eichhornia crassipes*, after introduction of two weevils (*Neochetina eichhorniae* in 1974 and *Neochetina bruchi* in 1975) and one pyralid (*Niphograpta albiguttalis* in 1979) (see arrows). (Center *et al.*, 2002.)



classical biological control programs. This could be because many weedy grasses have close relatives among the grasses grown in agriculture as crops and with their simple architecture, grasses have fewer herbivores per grass species. Scientists have steered clear of targeting grasses for biological control to avoid any problems if the natural enemies introduced were not specific enough. However, now two different biological control programs are under way in the USA to control weedy grasses, with specificity of agents being carefully evaluated.

Annual weeds of arable agriculture are also not often targeted for biological control. These plants have a short life span and occur in environments unstable for establishment and persistence of natural enemies. In addition, biological control of weeds requires a period of time for establishment and increase of the natural enemy before control is evident and normal cropping practices do not allow for such a slow response.

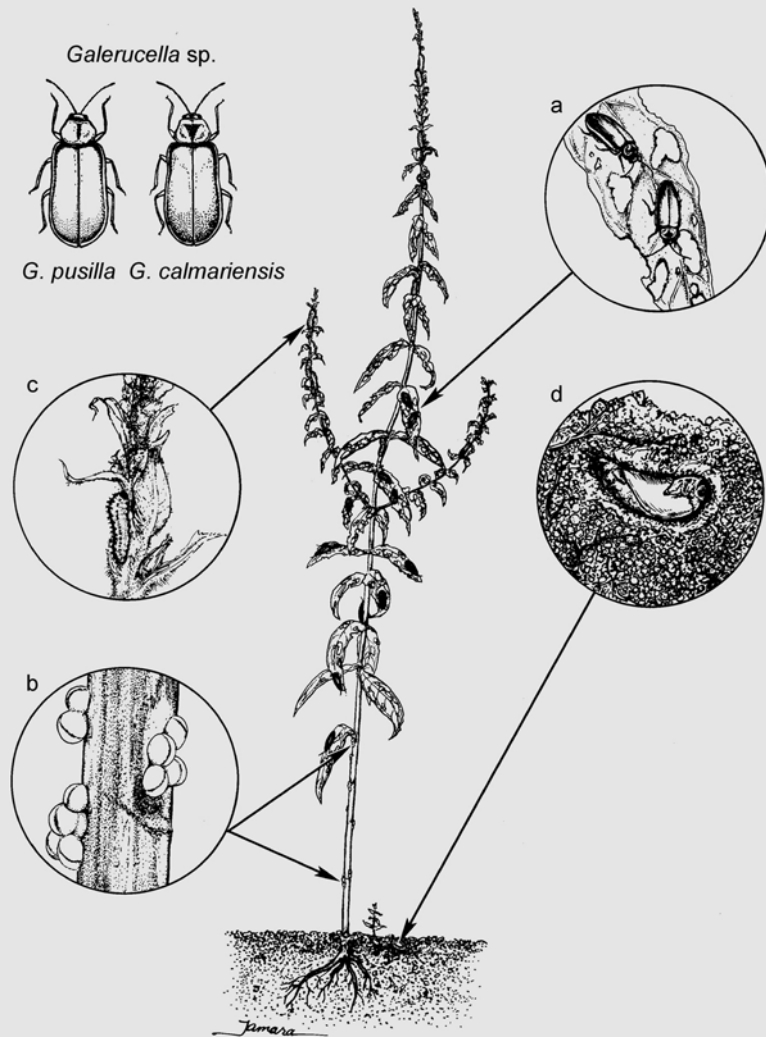
Invertebrate natural enemies

Based on a 1992 summary, the beetles are the order of insects most frequently utilized, with 30% of the species used providing control (Table 14.1). In comparison, the next most frequently used orders, moths and butterflies (Lepidoptera) and bugs, aphids, and scales (Hemiptera), produced successful control in 18 and 42% of cases, respectively. Although this figure for Hemiptera was high, it is based on only 19 species released, while for beetles (Coleoptera), 109 species had been used. Among the beetles, the most successful families were the leaf-feeding beetles (Chrysomelidae) and the weevils (Curculionidae), providing control in 31 and 39% of cases, respectively (see Box 14.1).

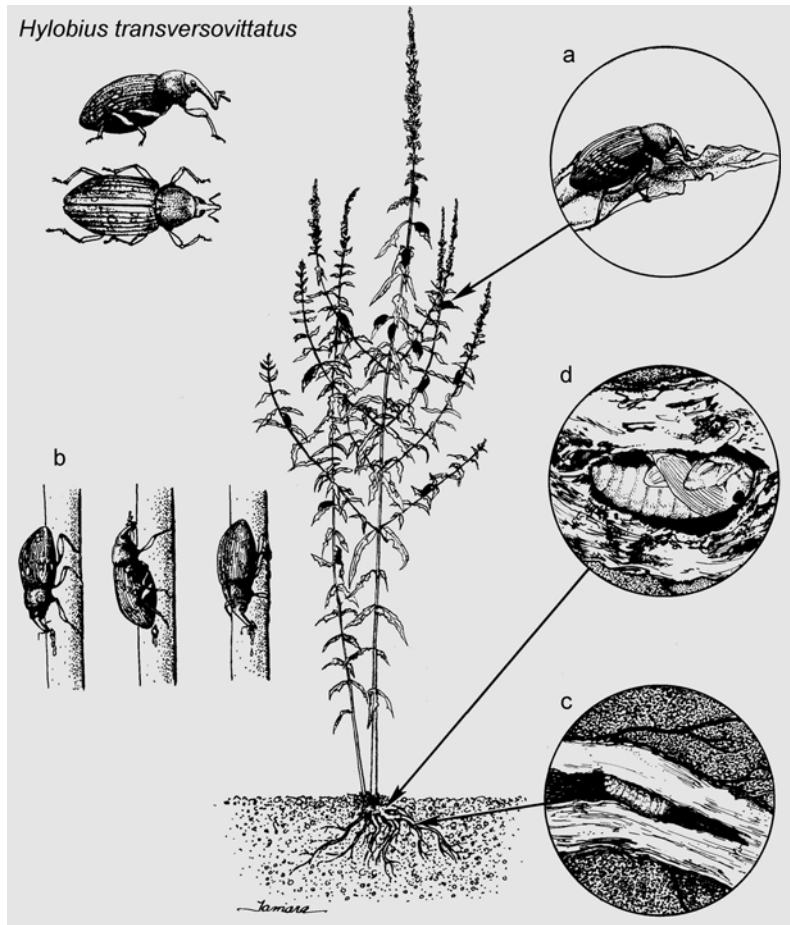
In a 1990 summation, the single most successful individual natural enemy was the scale insect *Dactylopius ceylonicus* which, along with some of its close relatives, has been repeatedly used to control prickly pear cactus in numerous parts of the world. These scale

Box 14.1 Releasing insects to control purple loosestrife in the United States

Purple loosestrife, *Lythrum salicaria*, is a lovely plant, creating large monotypic stands with tall purple flower heads covering wetlands and lining roadside ditches in North America. This plant is attractive but it is not native, having invaded from Europe. It reduces the biotic diversity of wetlands by outcompeting the native plant species and altering the wetland nutrient cycling. The natural food and cover used by



Life cycle of the leaf beetles *Galerucella californiensis* and *Galerucella pusilla*. a. Adults emerge in spring and feed on newly formed leaves of purple loosestrife. b. Females lay eggs for approximately 2 months in spring; batches of two to ten eggs are laid daily on the plant stem or in leaf axils. c. Developing larvae feed extensively on buds, leaves, and stems. d. Pupation is in the soil or litter near the host plant. Adults are short lived, dying soon after the spring egg-laying period. (Drawing by Tamara Sayre; Malecki et al., 1993.)



Life cycle of the root weevil *Hylobius transversovittatus*. a. Adults emerge in spring and feed nocturnally on new leaves of *Lythrum salicaria*. b. Females lay eggs over 2–3 months, individually depositing 1–3 eggs into the stem each day and covering them with frass. c. Developing larvae mine extensively in the roots. d. Mature larvae form a pupation chamber in the upper parts of the roots, emerging as adults in late summer or the next spring. Adults can live for several years. (Drawing by Tamara Sayre; Malecki et al., 1993.)

the specialized wetland birds including black terns (*Chlidonias niger*), least bitterns (*Ixobrychus exilis*), pied-billed grebes (*Podilymbus podiceps*) and marsh wrens (*Cistothorus palustris*) have been altered by invasion of purple loosestrife (Blossey et al., 2001). Many have called plants like purple loosestrife “environmental weeds,” because their impact is in displacing the native vegetation. However, in this case, purple loosestrife is also unpalatable to livestock and it has caused agricultural losses by shrinking wetland pastures and hay meadows.

Early in the nineteenth century, this plant was brought from Europe to North America accidentally during shipping but also purposefully, as a medicinal herb to treat diarrhea, dysentery, bleeding, wounds, ulcers, and sores. It became well established along the coast in New England by 1830 and was already spreading. It now occurs through much of the northeastern and midwestern USA, southeastern

Canada and other scattered locations to the west. There are no effective methods for controlling purple loosestrife using cultural methods or herbicides. Therefore, biological control efforts began in 1985. A total of 120 species of phytophagous insects associated with purple loosestrife were collected by Bernd Blossey in Europe but only 14 were considered host specific. Work has continued with four species: two leaf beetles, a root-mining weevil and a flower-feeding weevil. It was predicted that releasing all of these species would enhance control and all species have therefore been established within the range of purple loosestrife in North America. The leaf beetles and root weevil both showed great promise. The leaf-feeding beetles were easier to rear and were known to increase to high densities when released in stands of purple loosestrife, so focus has been on distribution of these species first while methods for mass-production of the root weevils were being developed.

Wetlands are scattered and can cover enormous areas, and Blossey found that natural dispersal of the beetles was slow. Methods for propagation and release of the leaf beetles were by far easiest to develop compared with the other natural enemies. Because purple loosestrife is not an agricultural weed, this control program then took on more of a grassroots approach, generated by land managers interested in protecting and preserving the natural flora and fauna. Questionnaires were sent to land managers in more than 30 states to gain an overview on opinions regarding use of classical biological control against purple loosestrife. State and federal agencies and private entities and schools became involved in rearing and releasing natural enemies and in monitoring their impact. By 2002, the leaf-feeding beetles, *Galerucella californiensis* and *G. pusilla* had been released in 33 states and more than 1500 wetlands in North America. In New York State alone, populations of *Galerucella* built up and spread with resulting decreases in purple loosestrife that have been spectacular in some areas. These results only provide a glimpse of the results based on 3–4 years of monitoring. Blossey estimates that it could take 10–20 years before results from this program can accurately be evaluated.

insects increase in numbers, covering plants and sucking the phloem sap. Their feeding often leaves openings for disease organisms to invade the fleshy cactus pads, leading to rot and death of the infected parts of the plant.

Using these data, the biological attributes needed for success are not crystal clear. However, biological factors associated with establishment of agents are high rate of increase, long-lived adults, numerous generations per year, and low feeding rate associated with small sizes of individuals (Crawley, 1989). Interestingly, many of the successful species are not uncommon in their native lands, as would be suggested by the enemy release hypothesis but rather are often widespread and abundant in their native homes (Bergelson & Crawley, 1989). As can be seen, success of a natural enemy cannot always be predicted according to the type of agent or the type of damage. This was certainly the case with a gall wasp (*Trichilogaster acaciaelongifoliae*) from Australia attacking an invasive leguminous tree, *Acacia longifolia*, in South Africa. Adult gall wasps were introduced, although they seemed

sluggish and there seemed little chance for success based on poor success with wasps and the fact that gall formers were not often known for significantly aiding control. Surprisingly, within a few years, the acacia branches were weighed down with galls and the infestation of this gall wasp had stopped seed production (Dennill, 1985).

Practically all types of phytophagous insects have been considered as biological control agents. While host specificity is the first concern, the most host-specific insects are not always used, in part because these species might be more challenging for handling, transport and rearing. Blossey and Hunt-Joshi (2003) compared the establishment and success of root feeders versus foliage feeders used for biological control of weeds. Perhaps because the activity of root feeders goes undetected without specific evaluation and because they are more difficult to rear and study, root feeders have been neglected for many years. There were only four releases of root feeders between 1902 and 1960 and, while this increased to 46 from 1960 through 1999, releases of aboveground feeders were almost 10 times as numerous. In the past 100 years, approximately the same percentages of root feeders became established after release as aboveground herbivores. However, a large difference was found in the percentages of established species contributing to control, with 53.7% for root feeders and 33.6% for aboveground feeders. Perhaps this recent summarization will encourage more biological control programs to spend the extra effort necessary to work with cryptic root feeders and re-examine emphasis on other types of invertebrates that are more difficult to work with. The question remains as to why root feeders seem to be more successful. One of the major reasons cited for lack of establishment and success of all phytophagous herbivores released for classical biological control is predation and parasitism. Certainly, root feeders would be more protected from aboveground predators and parasitoids in their underground homes so perhaps this could explain why their survival is better, at least in part. However, we know that living in the soil certainly does not always translate into complete protection from natural enemies but would instead result in exposure to soil-dwelling natural enemies.

By far the greatest successes have occurred when using introduced agents against introduced weeds (Goeden & Andrés, 1999). Only four species of native weeds have been successfully controlled using intentionally introduced exotic herbivores and all four of these weeds belong to the prickly pear cactus genus *Opuntia*.

International cooperation

Biological control of weeds has been used around the world, with releases in 75 different countries (Table 3.1). In fact, many plant species that are weeds in one country have also been introduced to numerous other countries where they have become troublesome. Cooperation among countries has often been excellent, with the result that once

a natural enemy has proven successful in one country, it has been released for weed control in numerous additional countries. However, the door swings both ways and we often find that a weed that is difficult to control in one country is similarly difficult to control elsewhere. Many programs have released agents against *Lantana camara* in different countries but with little success, except in Hawaii (McFadyen, 1998). In this case, the international cooperation leading to releasing similarly unsuccessful agents again and again in different countries has decreased the overall success rate of biological control of weeds.

14.3.2 Commercial availability and release

Classical biological control releases are conducted by governmental or academic organizations. The goal is to release each natural enemy so that it will spread and increase on its own. However, resources for rearing and releasing natural enemies are limited. Some natural enemies do not disperse quickly, or at least not quickly enough for land managers wanting to get rid of weeds. Therefore, in the USA small companies have been developed to provide the same phytophagous insects that have previously been released through classical biological control programs. As one example, one US company markets 22 natural enemies, predominantly beetles, a few caterpillars and one stem gall-inhabiting fly, for use against 10 weed species.

In some cases, natural enemies may already be present in an area of concern but growers want to boost populations to improve or hasten control; this would then be inoculative augmentation. Such augmentation would certainly be necessary if insecticides had been applied to the land, killing natural enemies that had previously been present. There is also the possibility that although the weed in question had been eliminated, with disturbance that same weed could recolonize the site and once more increase in abundance. In such a case, land managers might want to release natural enemies.

14.3.3 Conservation

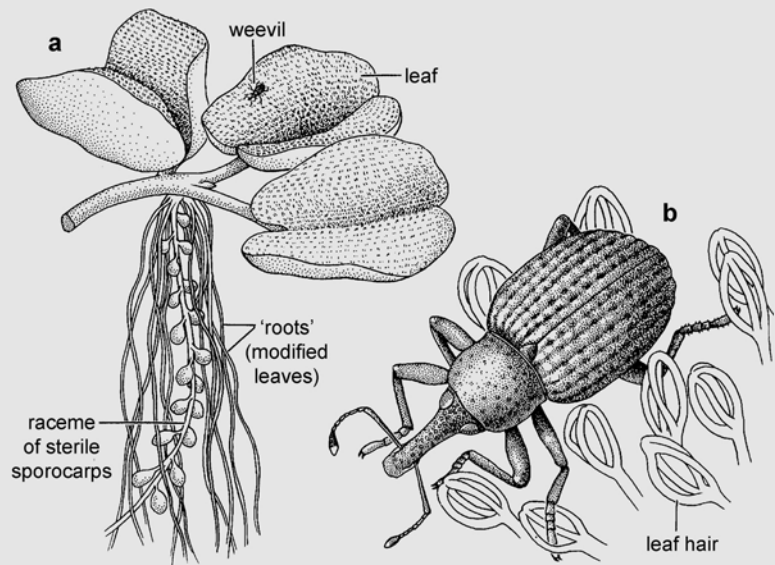
Conservation biological control strategies have not been used widely for weed control yet interest in this type of control is growing. Conservation biological control of weeds using invertebrates has taken two major forms. Most programs are based on directly enhancing the efficacy of introduced natural enemies that are released. Of course, knowledge of the factors limiting populations of natural enemies is critical before being able to facilitate an increase in the herbivore population and for this reason, conservation tactics are tailored to specific systems. One well-known method for improving the efficacy of some natural enemies is manipulating plant quality (Newman *et al.*, 1998). Nitrogen fertilization of weeds seems counter-intuitive but this has improved control of both prickly pear (Box 13.1) and *Salvinia* (Box 14.2) by the herbivorous insects released against them.

Box 14.2 | The aquatic weed *Salvinia molesta*

Salvinia molesta is a free-floating aquatic fern originating in southeastern Brazil. It is a botanical curiosity and has been used extensively as an aquarium plant and thus became distributed throughout the tropics over the past 50 years. Unexpectedly, if introduced to waterways, it often readily became established and could cause major problems. This plant grows very fast, doubling in size every 2.2 days and it has grown to cover lakes and rivers with mats 1 m thick. With a single layer of plants, canoes cannot pass while with a thicker mat, diesel-powered boats cannot pass. It also obscures access to the water for domestic animals and humans. After this weed became a serious problem in many different countries, control efforts were initiated in the 1940s.

Salvinia created the most problems in areas where the human inhabitants relied heavily on waterways for transportation. The floodplain of the Sepik River in Papua New Guinea is just such a place where 80,000 people depend on transportation across the water for most goods and services. In addition, a staple food is made from the pith of sago palms and transport over water is essential for harvesting and distribution of palms. *Salvinia* became such a problem in this area that the lives and livelihoods of the inhabitants were threatened. At first, control was attempted with herbicides and removal and containment using a boom in the water, but these methods were not successful on a long-term basis.

In 1959, the *Salvinia* occurring on Lake Kariba on the Zambia/Zimbabwe border was identified as *Salvinia auriculata*. Therefore, the native home for this species, South America, was searched for natural enemies (Thomas & Room, 1986). A moth, a grasshopper, and a weevil were collected and introduced to various countries, but these agents often did not establish or established only very poorly and



The aquatic weed *Salvinia molesta* and the small black weevil *Cyrtobagous salviniae* from southeastern Brazil, which was successfully released for classical biological control. (Illustration by Karina H. McInnes, Gullan & Cranston, 2000.)

provided no control. By 1970, researchers realized that the cause of the problem was not *S. auriculata*, and the problematic weed was instead identified as *S. molesta*. In the native area for *S. molesta*, southeastern Brazil, the same three species of natural enemies were thought to be attacking *S. molesta*. However, further investigations showed that the weevil in southeastern Brazil was really a new species (*Cyrtobagous salviniae*) and an excellent biological control prospect as well. The 2 mm long black adult *C. salviniae* feed selectively on growing points of *Salvinia*. *Salvinia* is very susceptible to this type of feeding because this plant relies on vegetative propagation. The tiny white weevil larvae tunnel through the rhizome, eating the vascular tissue, and the total effect of these weevils is devastating to *Salvinia*. These weevils are host specific, are very efficient at finding host plants, and can live at high densities; all of these are great characteristics for an effective phytophagous insect for weed control.



a. A creek in temperate Australia, near Sydney, infested with *Salvinia molesta*. b. This same creek 3 years after the salvinia weevil, *Cyrtobagous salviniae*, had been released. (Photos by M. Julien.)

This weevil was first introduced to Lake Moondarra in Australia. Within only 14 months, *Salvinia* was completely gone from the 200 infested hectares. With such stupendous results, the weevil was then introduced to many additional locations, including the Sepik River floodplain. Releases to control the Sepik infestation began in May 1983 and, by August 1985, the area of lake covered by *Salvinia* had been reduced from 250 km² to 2 km²; this weevil had destroyed 2 million tons of *Salvinia* in slightly over 2 years. During this program, researchers learned that if the plants were low in nitrogen, the beetles did not reproduce well. Therefore, urea was added to the water to enhance establishment and activity of the weevils. In the Sepik River floodplain, and in Australia, the control achieved was cost effective, environmentally sound, and permanent. The weevil has been introduced to additional areas where control was excellent. In one study in Sri Lanka, a cost : benefit analysis indicated returns on investments of 1 : 53 in terms of cash and 1 : 1678 in terms of hours of labor. In recognition of the benefits derived from the ecological research resulting in biological control of *Salvinia*, the team responsible for this introduction was awarded the UNESCO Science Prize in 1985.

The use of pesticides may interfere with the first priority in biological control introductions, establishment of control agents. In some cases, insecticides being used to control insect pests can disrupt weed control by herbivorous insects. In South Africa, a weevil, *Trichapion lativentre*, is able to control the leguminous weed *Sesbania punicea* but is very sensitive to insecticides used in citrus orchards to control insect pests (Hoffmann & Moran, 1995). Drift from citrus orchards can cause declines in weevil populations during the summer and the weed then can reach high densities up to several meters from orchards. Clearly, controlling insecticide applications could conserve populations of this natural enemy. On the other hand, insecticides have been used in South Africa to help conserve a natural enemy. The scale insect *Dactylopius* sp. was not providing control of *Opuntia* in specific areas where two species of lady beetles that feed on this scale were abundant. Insecticides were applied at low rates to reduce predator populations but not kill the scales, thus enabling control of the prickly pear by the scale (Annecke *et al.*, 1969).

14.4 | Vertebrates

Larger vertebrates can be used for weed control but this is never the sole use of these animals and there are certainly drawbacks to this approach. These animals do not specifically feed on only one plant species and their activity is generally useless for controlling weeds unpalatable to livestock. Regardless, sheep and goats have been used to control tansy ragwort and blackberry, and geese have been used to control weeds in cotton (Julien & White, 1997). However, fish are the major group of vertebrates that have been used quite extensively for weed control.

Herbivorous fish also do not feed on only one plant species but usually graze on a diversity of aquatic plants. Eleven species of fish have been used to control aquatic weeds and algae, although by far the most commonly used species is the grass carp (*Ctenopharyngodon idella*). This species is native to the Amur River in Asia and prefers moving rather than still water. Grass carp, also called white amurs, can grow to up to 40 pounds and they live for over 10 years. They are omnivorous when very young but, after the 8–12-inch (20–30 cm) stage that is released for weed control, they are strict vegetarians. They eat a wide variety of plants but are selective, preferring softer, submerged plants and they are not effective against floating plants. They prefer not to feed in areas with regular human activity, such as boat docks or swimming areas. Grass carp have been released for weed control in at least 36 countries, including the USA (Julien, 1992).

In the USA, there is concern regarding whether this species will negatively affect native species of fish. Although no direct negative influence has been established, grass carp can alter the aquatic habitat, potentially making it unacceptable for native fish species. Therefore, grass carp are released only in confined bodies of water, especially those not connecting with other bodies of water during floods. Release of grass carp is not permitted by all states and, in the USA most grass carp that are released have been sterilized so that they will not reproduce. Grass carp are raised in fish hatcheries and their eggs are subjected to temperature or pressure shock so that an extra chromosome is present, making the eggs triploid instead of diploid. Triploid grass carp are sterile, thus, although the individuals released can live a long time, they will not reproduce. As an extra precaution, sterilized grass carp are tested to insure that the sterilization treatment was effective and only triploid fish are released.

Use of grass carp to control aquatic vegetation can be very effective but it can require several years before an effect is seen. The correct number of fish must be released so overgrazing does not occur. It is important that grass carp are released where the weeds that must be controlled are the species preferred by grass carp – grass carp do not control algae or floating, surface plants. Thus, grass carp can offer an inexpensive long-term solution to difficult problems with aquatic weeds although they are certainly not the answer for all situations.

FURTHER READING

- Bellows, T. S. & Headrick, D. H. Arthropods and vertebrates in biological control of plants. In *Handbook of Biological Control*, ed. T. S. Bellows & T. W. Fisher, pp. 505–516. San Diego, CA: Academic Press, 1999.
- Blossey, B. & Hunt-Joshi, T. R. Belowground herbivory by insects: influence on plants and aboveground herbivores. *Annual Review of Entomology*, **48** (2003), 521–547.
- Goeden, R. D. & Andrés, L. A. Biological control of weeds in terrestrial and aquatic environments. In *Handbook of Biological Control*, ed. T. S. Bellows & T. W. Fisher, pp. 871–890. San Diego, CA: Academic Press, 1999.

- McFadyen, R. E. C. Biological control of weeds. *Annual Review of Entomology*, **43** (1998), 369–393.
- Myers, J. & Bazely, D. *Ecology and Control of Introduced Plants*. Cambridge: Cambridge University Press, 2003.
- Schroeder, D. Biological control of weeds. In *Recent Advances in Weed Control*, ed. W. E. Fletcher, pp. 41–78. Farnham Royal, Slough, UK: Commonwealth Agricultural Bureau, 1983.
- Wapshire, A. J., Delfosse, E. S. & Cullen, J. M. Recent developments in biological control of weeds. *Crop Protection*, **8** (1989), 227–250.

Plant pathogens for controlling weeds

Just as microorganisms can decimate populations of insect pests, some microbe species are very effective at growing and reproducing using living plants as nutrient sources. Plant pathology is the study of microbes causing plant disease, with emphasis on how to control these microbes and manage their populations. However, some plant pathologists use their training to investigate the use of living microorganisms to control weedy plants. These plant pathologists are working to increase populations of plant pathogenic microbes, quite the opposite from the normal activities of plant pathologists.

Most major categories of microscopic organisms, viruses, bacteria, fungi, protists (a heterogeneous group of single-celled organisms), and nematodes, have members that are plant pathogens. However, the microorganisms most commonly selected for control of weeds are the fungi. As we will discuss, use of microbes for biological control of weeds has focused both on classical and inundative approaches to biological control. Different groups of fungi with very different attributes have been utilized for these very different biological control approaches.

15.1 Inundative biological control

The main goal of an inundative release is to attack an entire weed population with a single application of a pathogen without the assumption that the natural enemy will persist. This application strategy is similar to use of chemical herbicides and, in fact, the same application equipment can often be used for applying microbes that is used for applying chemical pesticides. Pathogens for weed control are generally applied annually, similar to applications of a chemical herbicide. Due to the similarities of these biopesticides with chemical herbicides, products are often called bioherbicides. Inundative biological control has employed fungi in most cases, so products are often called mycoherbicides (the prefix “myco” refers to fungi). Of course, these products differ significantly from synthetic chemical pesticides. In particular, they are highly host specific and only target

one species of weed, while chemical herbicides would affect many different species of plants. Also, they must be handled to make sure that the organisms remain viable.

Almost all of the pathogens developed to date for inundative application are fungi that are easy to mass-produce on inexpensive media. No matter where and when these pathogens are applied, it must be remembered that these are living organisms. Fungal spores, the active ingredient in most products, are generally sensitive to ultraviolet radiation and require free moisture (such as dew) to cause infection. Some products therefore have been improved to reduce spore desiccation.

In the late 1960s and during the 1970s, two products were developed and registered with the US Environmental Protection Agency. These still provide landmark examples demonstrating that such products can be effective. Both of these products were developed for niche markets and have remained for sale since the early 1980s.

Box 15.1 | A divine bioherbicide

Milkweed vine (*Morrenia odorata*) was introduced to Florida from South America as an ornamental plant, but it became a major problem when it began competing with trees in citrus orchards. This plant more commonly became known as strangervine because it girdles tree limbs, competes for water, sunlight, and nutrients, and interferes with harvesting, spraying, and irrigation. Controlling this vine in the 120,000 hectares it infested required mechanical means or herbicides. These efforts were not always successful and they were expensive, costing about \$124 per hectare every year.

A pathogen, *Phytophthora palmivora*, was isolated from diseased and dead strangervines in 1972 in Orange County, Florida. This pathogen belongs in the Chromista, or water molds. Species in this group of pathogens can cause rot of stems and roots. Small-scale field tests yielded 96% mortality of vines within 10 weeks after applying the pathogen to the soil in an orchard. Researchers were optimistic that this fungus could be used effectively to control strangervine but they needed to confirm that it would not infect crop plants. In greenhouse tests, however, this pathogen could infect seedlings of onion, cantalope, watermelon, and citrus before they emerged and seedlings of pea, squash, and watermelon after emergence (TeBeest *et al.*, 1992). However, researchers reasoned that laboratory conditions where this pathogen was applied at high concentrations in a sterile environment were not comparable to field situations. In addition, the citrus orchards where this pathogen was to be applied are monocultures and these other potentially susceptible plants are not present.

In 1981, *P. palmivora* was the first pathogen registered for inundative control against weeds in the USA. The product that is marketed was named DeVine® and is composed of the longer-lived spores of this pathogen, in a liquid concentrate for spray application directly to strangervine. It can kill both seedling and mature strangervine plants and regularly yields 90% control. This pathogen cannot be applied where the susceptible vegetables are grown in Florida (Charudattan, 1991). Activity of this pathogen persists for several years if areas where it is applied are not disturbed. This is beneficial for growers but creates difficulties for the company producing this pathogen because there is not a regular demand every year.

Table 15.1 | Plant pathogens for inundative biological control that have been registered and are being mass produced

Plant pathogen (product name)	Weed	Target sites	Country
<i>Bacteria</i>			
<i>Xanthomonas campestris</i> pv. <i>poae</i> (Camperico)	Annual bluegrass (<i>Poa annua</i>)	Golf courses, turf	Japan
<i>Fungi</i>			
<i>Chondrostereum purpureum</i> (BioChon)	Broad-leaved trees	Tree plantations	USA
<i>Colletotrichum gloeosporioides</i> (Biomal)	Mallows (<i>Malva</i> spp.)	Various crops	USA, Canada
<i>Colletotrichum gloeosporioides</i> (Hakatak)	Silky needlebush (<i>Hakea sericea</i>)	Natural areas	South Africa
<i>Colletotrichum gloeosporioides</i> f.sp. <i>aeschynomene</i> (Collego)	Northern jointvetch (<i>Aeschynomene virginica</i>)	Rice and soybean	USA
<i>Cylindrobasidium laeve</i> (Stumpout)	Acacias (<i>Acacia</i> spp.)	Tree plantations	South Africa
<i>Chromista</i> (previously <i>Oomycetes</i>)			
<i>Phytophthora palmivora</i> (DeVine)	Stranglervine (<i>Morrenia odorata</i>)	Citrus	USA

After Charudattan, 2001.

DeVine® was developed for control of stranglervine, an important weed in citrus orchards in Florida (Box 15.1). Collego® was developed to control northern jointvetch (*Aeschynomene virginica*) in rice and soybeans in the southeastern United States. Since these two products were developed, five more products for inundative release have been registered for use in a diversity of systems (Table 15.1). All of these products are intended for very specific purposes. For example, BioChon and Stumpout are both based on wood-inhabiting fungi that are applied to cut stumps to prevent the stumps from resprouting (Fig. 15.1). Both of these products rot unwanted tree stumps in tree plantations and can kill unwanted weedy invasive trees and shrubs. The bacterium in Camperico is intended to remove annual bluegrass (*Poa annua*) and is applied immediately after golf course grasses have been mowed.

There is definite interest in increasing the number of bioherbicides on the market. Recently, 53 pathogens were listed as showing promise as weed control agents in 41 different situations (Charudattan, 2001). However, especially with fungal pathogens of weeds, it is a challenge for companies to manufacture a product with a long shelf life. In contrast, the chromist (previously an oomycete, a fungal relative) *Phytophthora palmivora* used in DeVine has been too effective. End-users have not purchased this product on a yearly basis because this fungus has a stage that persists in undisturbed field sites long

Fig. 15.1 The basidiomycete fungus *Chondrostereum purpureum*, contained in the product BioChon, producing fruiting bodies on an infected branch. A suspension of this saprophytic fungus is applied to stumps of weedy trees to prevent resprouting. This fungus was initially used to control American black cherry (*Prunus serotina*) in Europe. (Photo courtesy of Clive Shirley.)



after application. Thus, the company producing this fungus has not been able to sell this product to the extent intended, because users do not buy it on a regular basis.

15.2 | Inoculative biological control

Some native or naturalized plant pathogens that might be effective for controlling native or naturalized weeds are not so easy to mass-produce. Therefore, an inundative approach is not possible for these species. Researchers have investigated whether augmenting populations of these pathogens that are already in the field could enhance weed control. In cases where such an inoculative release strategy has been investigated, the pathogen has often been gathered in the field, stored, and then applied. Inoculative biological control of weeds with plant pathogens has been tested for several different systems but is really not yet being used operationally.

A good example of an inoculative release involves the rust fungus *Puccinia canaliculata*, which infects yellow nutsedge, one of the worst weeds worldwide (Fig. 15.2). Epiphytotics (epidemics of plant pathogens) of this virulent pathogen occur naturally in August or September. If this fungus is released early in spring, it attacks plants much earlier than it normally would and flowering and tuber formation by nutsedge are therefore reduced. A product containing this fungus, called Dr. BioSedge, has been developed and registered for sale. Unfortunately, the stumbling block has been availability of the product because mass-production of this obligate pathogen is difficult and therefore relatively expensive.

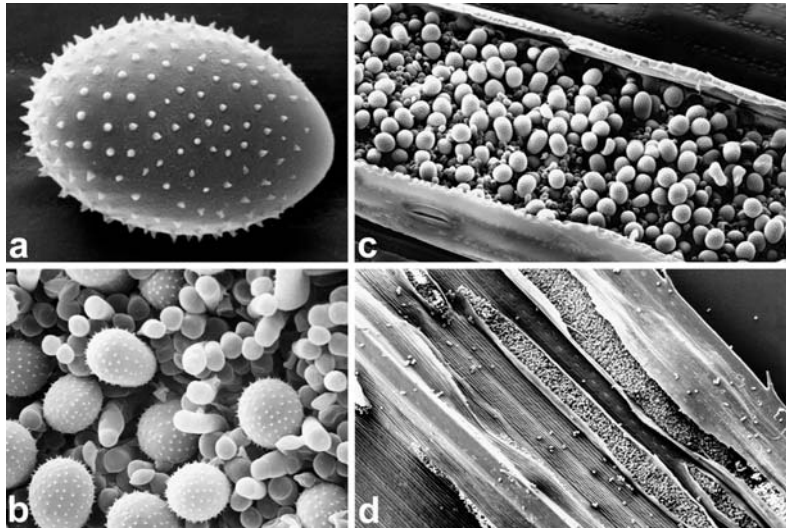


Fig. 15.2 The rust fungus *Puccinia canaliculata* infecting the weed yellow nutsedge, *Cyperus esculentus*. a. Urediniospore that will be wind-borne. b and c. Urediniospores being produced from a leaf of yellow nutsedge. d. Severe infection with urediniospores being produced in eruptions on the leaf surface, parallel with the main axis of the leaf blade. (Wetzstein & Phatak, 1987.)

15.3 | Classical biological control

Introduction of exotic herbivorous arthropods has been successful for controlling a myriad of weed species. This same approach has also been tried with fungal pathogens. A major requirement for such programs when herbivorous arthropods are chosen as biological control agents is to make certain the agent is very host specific. Although there are numerous groups of fungi that are host specific, the group used extensively for classical biological control is the rust fungi. Rusts can be very damaging to their hosts and are also usually highly host specific.

Rust fungi are actually quite closely related to mushrooms based on fruiting structures and spore forms (both belonging to the Basidiomycetes), but to the human eye they do not look like mushrooms at all. Much of their growth occurs within hosts. Rusts can have complicated life cycles with up to five different types of fruiting structures and five different types of spores that occur in a specific sequence. Although some rusts require two different species of hosts in their life cycle, rusts considered for biological control have only one host species; they are autoecious. Rust fungi are obligate pathogens in nature and only some have been grown in the laboratory on special media.

Rusts infect plants by entering through stomates, the microscopic openings in the plant epidermis used for gas exchange. Infected plants develop rust-colored spots on leaves and stems where the rust spores are produced. These areas, called lesions, can become black toward the end of the season when more persistent spores are produced. In severe cases, infected plants become weakened and stunted and can even be killed. Rust spores are principally spread on the wind, an

Table 15.2 Successful classical biological control introductions of microbes for weed control		
Pathogen	Target weed	Country of introduction
Fungi ¹		
Rusts		
<i>Puccinia chondrillina</i>	Rush skeletonweed (<i>Chondrilla juncea</i>)	Australia and continental USA
<i>Puccinia carduorum</i>	Nodding (musk) thistle (<i>Carduus nutans</i>) ²	Continental USA
<i>Phragmidium violaceum</i>	Blackberry (<i>Rubus</i> spp.)	Chile
<i>Uromycladium tepperianum</i>	<i>Acacia saligna</i>	South Africa
Smuts		
<i>Entyloma ageratinae</i>	Hamakua pamakani (<i>Ageratina riparia</i>)	USA (Hawaii)

¹*C. purpureum* and *C. laeve* belong to the Basidiomycota; *Colletotrichum* spp. belong in the Deuteromycetes.
² Success was in combination with three species of herbivorous beetle that were also part of the classical biological control program against nodding thistle (Kok, 2001).
After Charudattan, 2001; Kok, 2001.

aspect that is very helpful in spreading these pathogens after they are introduced. The capacity of rusts to spread readily is especially important because rust inoculum is not easily mass-produced.

How are rusts for classical biological control found? Because the symptoms of rusts are often quite evident, they are relatively well known and described in the literature. This makes it easier during foreign exploration because scientists know whether a rust species is associated with a certain weed. Microbes are sensitive to environmental conditions and they generally cannot seek shelter to protect themselves if conditions become harmful, for example if free moisture is not available or temperatures are either too high or too low. Therefore, for classical biological programs, it is important to understand the environmental needs of the rust being considered for classical biological control and compare these needs to the environment in the release area. To release rusts, spores in water are sprayed onto plants or spores are dusted onto plants. Alternatively, harvested infected host plant parts are released in the field. After the rust becomes established in the weed population, it will spread on its own.

The first plant pathogen used for classical biological control of weeds was the rust fungus *Puccinia chondrillina*, successfully released into Australia in 1971 (Box 15.2). This species has also been used in the USA since 1976. New pathogens are being developed for classical biological control of weeds, and this has resulted in several additional successes (Table 15.2). Most of these releases involve obligate pathogens that, without human intervention, increase on their own within the host population. Almost all of the successful programs have used rust and smut fungi.

Box 15.2 | Introducing a rust to control rush skeletonweed in Australia

The first example of use of a pathogen for weed control in any country was the introduction of a rust to control rush skeletonweed (*Chondrilla juncea*). The success of this program boded well for this new type of control. Rush skeletonweed is an important weed in agricultural areas in both southeastern Australia and California, where it was introduced from mediterranean Europe. A rust fungus, *Puccinia chondrillina*, attacks skeletonweed in areas where it is native and, in fact, different strains of the fungus are quite host specific. It was found that fungal strains from near Vieste, Italy, and from France were most virulent against the strain of this weed that had been introduced to Australia and California, respectively. The rust strain from Italy was introduced to Australia and it spread rapidly, up to 80, 160, and 320 km from the release site within the 8th, 10th and 12th generations, respectively. Infections reduced plant height, the number of flowers, and the number of viable seeds. Heavily infected plants died before producing flowers, especially in areas with climates favorable for infection. In such areas in Australia, plant mortality has exceeded 90%.

In California, results are different and instead, this fungus suppresses rush skeletonweed. The northern California populations of skeletonweed were reduced after this rust was introduced along with two arthropod natural enemies. Of the three agents, the rust appeared to be the most effective agent, reducing skeletonweed populations by 56–87%. In the areas where these studies were conducted, densities of skeletonweed are similar to the normal levels found in areas where this weed is native.

Since the first release occurred in only 1971, use of pathogens is a relatively new approach for classical biological control of weeds, considering that the first release of an arthropod to control a weed occurred in 1795 (Goeden & Andrés, 1999). Interestingly, this later development of plant pathogens for classical biological control of weeds is similar to the lag in use of pathogens for controlling arthropods, when compared with the earlier and greater efforts in use of arthropod natural enemies.

FURTHER READING

- Charudattan, R. Biological control of weeds by means of plant pathogens: significance for integrated weed management in modern agro-ecology. *BioControl*, **46** (2001), 229–260.
- Evans, H. C., Fröhlich, J. & Shamoun, S. F. Biological control of weeds. In *Bio-Exploitation of Filamentous Fungi*, ed. S. B. Pointing & K. D. Hyde, pp. 349–401. Hong Kong: Fungal Diversity Press, 2001.
- Evans, H. C., Greaves, M. P. & Watson, A. I. Fungal biocontrol agents of weeds. In *Fungi as BioControl Agents: Progress, Problems and Pitfalls*, ed. T. M. Butt, C. Jackson & N. Magan, pp. 169–192. Wallingford, UK: CABI Publishing, 2001.
- Roskopf, E. N., Charudattan, R. & Kadir, J. B. Use of plant pathogens in weed control. In *Handbook of Biological Control*, ed. T. S. Bellows & T. W. Fisher, pp. 891–918. San Diego, CA: Academic Press, 1999.

Part IV

Biological control of plant pathogens and plant parasitic nematodes

Biological control of plant pathogens and plant parasitic nematodes occurs at a different size scale than control of pestiferous invertebrates, vertebrates, or plants. Most plant pathogens are microscopic organisms, also called microorganisms or microbes, and they predominantly belong to the fungi, bacteria, and viruses, with some flagellate protists, among others. Plant pathogenic nematodes are generally covered within the context of plant pathogens, because the majority are microscopic. However, plant parasitic nematodes are more complex organisms than microbial plant pathogens, being eukaryotic and multicellular with organ systems.

Plant pathogens cause losses in agriculture, horticulture, and forestry. It was estimated in 1997 that in the USA alone, crop losses valued at \$9.1 billion due to diseases occur each year (Agrios, 1997). Losses to disease in developing countries are much greater and one could argue that these countries would be more sensitive to losses in food crops than the USA. Crop losses by plant parasitic nematodes were estimated in 1987 at US\$77 billion worldwide yearly on 21 crops (Sasser & Freckman, 1987).

Numerous plant pathogens have developed methods for rapidly infecting and dispersing, sometimes causing epidemics leading to serious levels of damage if not complete loss of a crop. In 1845 and 1846, an epidemic of the chromist *Phytophthora infestans* obliterated the potato crop, the staple food of the Irish populace, and caused widespread famine. This well-known example demonstrates the impact a plant disease epidemic can potentially have. As well as causing decreased yield, some plants attacked by plant pathogens should not

be eaten because their tissues become poisonous to humans and wild or domestic animals.

It is in the realm of crop protection that the majority of investigations of plant pathogens has been conducted. However, in native ecosystems, plant pathogens can also have serious impacts on ecosystem composition and dynamics. For example, introduced pathogens causing Dutch elm disease and chestnut blight altered the tree species composition of northeastern and midwestern North American forests by removing the American elm (*Ulmus americana*) and chestnut (*Castanea dentata*), respectively.

Biology and ecology of antagonists

16.1 | Types of plant pathogens and their antagonists

The term disease, in plant pathology, is defined as the malfunctioning of plant cells and tissues, resulting from continuous irritation and leading to development of symptoms. Plant diseases can be caused by several types of microorganism but the most frequently encountered and diverse are the fungi, bacteria, and viruses. Plant pathogens vary in the intensity of their relations with host plants from parasites, requiring the host to live, to saprophytes, which feed on dead organic matter. They range in their use of host tissues from being biotrophs, which feed on living cells, to necrotrophs, which kill plant tissues before ingesting their contents. Some plant pathogens are obligate parasites and can only grow and multiply in nature when living on or in living organisms (so these are all biotrophs). These include viruses, powdery and downy mildews, rust fungi, and some bacteria, among others. Other plant pathogens, including many fungi and bacteria, can live on dead as well as living plant material and these are therefore facultative parasites.

Both fungal and bacterial plant pathogens exist in the environment inside or outside of plants or in or on dead plant material. Many pathogens have active epiphytic (occurring on the plant surface without causing infection) and saprophytic phases in their life cycles. Some species have special persistent stages that live many years. Dispersal of bacteria and fungi is by wind and rain and sometimes they are carried by higher organisms. In contrast, plant pathogenic viruses live within plant cells and often depend on higher organisms, frequently insects, for dispersal or are transferred from plant to plant when plant roots grow together. Plant parasitic nematodes are soil-dwelling and attack plant roots. Some nematode species live within specialized structures on roots that offer protection and the common species are appropriately named cyst nematodes or root-knot nematodes.

Plant pathogens attack plant parts above the ground (leaves, stems, fruits, and flowers), at the soil surface, as well as in the soil (roots) and are said to have infected the plant only after microbes have

entered the host plant's tissues. The site of entry is often called the infection court. The majority of studies in biological control of plant pathogens has focused on diseases caused by pathogens in the soil. Soil-borne plant pathogens are especially well known for causing serious problems with germinating seeds and young, tender plants. For all plant parts, both aerial and roots, wounds provide a site for entry of pathogens into plant tissues. Thus, tissues that are damaged during growth, manipulation, or harvest are particularly susceptible to infection. Several major groups of plant pathogens, including viruses and phytoplasmas, are almost entirely dependent on wounds to gain entry to hosts. Another group of plant pathogens dependent on wounds are those fungi causing post-harvest diseases of fruit. After plant products are harvested, plant pathogens can cause serious damage during storage. Of particular concern are those saprophytic bacteria and fungi that are experts at rapidly colonizing organic matter, such as stored crops, to use as a source of nutrients.

Plant pathologists commonly call those microorganisms that suppress plant disease antagonists rather than natural enemies. The term antagonist is in many ways more appropriate for this field because these organisms frequently do not act directly to kill plant pathogens but instead often operate through a variety of more indirect mechanisms. Many antagonists belong to the same major taxonomic groups as many plant pathogens, the fungi and bacteria, and occupy the same habitats as the pathogens. Yet, they do not cause plant disease and instead can suppress disease.

Disease suppression in some cases is due to interactions between antagonists and plant pathogenic microorganisms. Some of these antagonists have developed life strategies of either feeding on or out-competing the primary pathogens. One strategy of antagonists is parasitism, frequently referred to as hyperparasitism because these antagonists are parasites of plant parasites. A second strategy used by antagonists is competition. Antagonists competing with plant pathogens are often specialized in their ability to exploit specific niches. Life histories of antagonists that impede the development of other microbes can be amazing in their complexity and diversity.

Disease suppression also can be due to induced resistance responses in the plant that are initiated by antagonists. In particular, this effect can be caused by viruses. Plants inoculated with viruses can develop resistance against further viral disease. In fact, many plant pathogens that are viruses are not considered as serious as fungal and bacterial pathogens because they seldom kill plants.

16.2 | Comparing macroecology with microecology

We have discussed the ecologies of natural enemies and pests that are both macroorganisms, as well as interactions between macroorganismal pests (e.g., animals and weeds) and microscopic natural enemies. To discuss the basis for biological control of plant pathogens, we now

switch to interactions between pests and their antagonists when both are microorganisms.

Studies of the interactions of plants and animals with their environment have dominated the general field of ecology. However, as early as 1934, George Gause published results from population dynamics studies using two species of ciliate protists, during which he found cyclic oscillations characteristic of predator/prey interactions. The use of microbes to investigate general ecological theories has once again become an active area of research only in more recent years.

The types of studies that have been and can readily be conducted with macroorganisms and microorganisms differ due to scale. Studies of the ecology of macroorganisms are dominated by investigations of associations using correlations to derive mechanistic explanations for observed patterns. This correlative approach is due to the complexity of larger organisms, their interactions with their surroundings, and, perhaps most importantly, the difficulty in conducting realistic experiments with them. In contrast, when studying the ecology of microorganisms, experimentation is used more extensively and frequently fairly realistic model systems constructed in the laboratory or greenhouse are used to investigate interactions.

The scale of ecological studies of microbes is more challenging than work with macroorganisms. Individual lady beetles or parasitoids can be observed as they attack individual pests. It is easy to count parasitoids and their host caterpillars and know how many individuals are present at certain times to evaluate changes in density, and to watch them as they interact. Studying relations among microbes is not so straightforward. A major explanation for these differences lies in the scale of an individual microorganism compared with a macroorganism. In fact, plant pathogenic microbes are seldom considered as individuals and are usually considered as groups; for example, the individual bacterial cells infecting a plant are considered together as one population. Interactions among colonies of microorganisms are not studied on the basis of individual cell by individual cell but rather population versus population. In some cases, for a plant pathogen to damage a plant or for an antagonist to deter a plant pathogen, an effect can be initiated by only one cell present in an auspicious location where it can readily reproduce. Alternatively, for some diseases it seems that many cells are necessary to initiate an infection successfully. However, plant disease does not occur due to one or a few microbial cells but only after those first cells reproduce, and many pathogen cells are required before disease symptoms are visible to the human eye. Likewise, effects due to antagonists are due to populations of cells. This group-based approach for infection by pathogens or protection by antagonists is supported by the fact that groups of microbes growing in the same microhabitat are often thought to be genetically identical, or clonal. For example, the fungal cells at one lesion on a plant are often genetically identical.

16.3 Ecology of plant pathogens and their antagonists

Antagonists are extremely diverse in their activity, making it difficult to summarize their relations with their respective environments. Perhaps because a “place to thrive” is critical for survival and growth of all pathogens and antagonists, ecological theories regarding the biological control of plant pathogens and plant parasitic nematodes have often emphasized concepts of niches (Campbell, 1989). All organisms occur in a niche, a concept including whatever that organism needs to live. Niches are theoretical constructs determined by the physiological properties of the organism, its environment and the resources needed by that organism. Because niches have been defined based on so many properties, they have often been described as “*n*-dimensional hypervolumes” (Hutchinson, 1957). The ideal conditions for an organism comprise its fundamental niche, while the conditions actually present are called the realized niche.

For microorganisms looking for a home, the vast majority of an aerial plant surface is a hostile environment, dry and with few nutrients. The roots of a plant provide different challenges due to the diverse community of competing organisms in the soil. Thus, at any location on a plant, microhabitats where moisture and nutrients are present, for example due to leakage from a wound or natural opening of the plant like a stomate, would present a valuable habitat for many microbes.

16.3.1 *r* and *K* strategies

Biological control of plant pathogens effected through competition is based on limiting the realized niches of pathogens due to the activity of other microorganisms. Among both plant pathogens and antagonists, some have high reproductive capacity and can quickly occupy a new resource (often called *r*-selected or ruderal species) (Andrews & Harris, 1986). These organisms are characteristic of disturbed sites where they can arrive and occupy a site quickly before other microbes arrive. At the other end of this spectrum are organisms that are characteristic of stable situations (*K*-selected species). These species are good at persisting and include both highly successful competitors and microbes that are stress tolerant. For example, while the opportunistic foliar fungal pathogen *Botrytis cinerea* colonizes new locations quickly (an *r*-selected species), it is a poor competitor when compared with *Penicillium* species, which produce secondary metabolites inhibiting potential competitors over a longer period of time (*K*-selected species). Of course, these are examples of extreme *r*- and *K*-selected species and, in reality, organisms occur along a continuum from *r* to *K*, with individual species often having mixtures of properties from both of these different life history strategies (Grime, 1977).

Plant pathogens occurring along this continuum include *r*-selected species that attack young or weakened plants (e.g., *Botrytis*,

Pythium, *Rhizoctonia*) but are not good at attacking healthy, well-established plants. In general, *r*-selected species are often not as good at competing with other microbes. In contrast, among the *K*-selected species, stress-tolerant species of plant pathogens grow slowly in marginal habitats where it is difficult to live. For example, rust fungi mostly grow externally in the dry, nutrient-poor environment of leaves where little competition occurs. Good competitors such as the *K*-selected wood-rotting fungi (e.g., *Armillaria mellea*) are often not quick at colonizing new sites but, once present, grow slowly and persist, while defending against secondary invaders.

r-selected species of microorganisms have proven to be successful agents for biological control in numerous instances. Although they are generally poor competitors, they are adapted to disturbed situations and can colonize new resources quickly. These species are also the easiest to mass-produce in the laboratory because growth requirements are often not restrictive. Due to their ability to colonize new habitats readily antagonists that are *r*-selected are often used protectively, with application occurring before infection by a plant pathogen might occur. After infection by a plant pathogen, biological control agents that are more competitive and those that are more persistent (*K*-selected species) are more appropriate for control. In practice though, most biological control agents are not used after a disease is well established but are more effective when used earlier, either before infection occurs or early in the course of an infection.

The ecology of a plant pathogen must be understood to develop a successful biological control strategy (Deacon, 1991). The conditions that are optimal for epiphytic or saprophytic growth of a pathogen must be similar to those that are optimal for its antagonists and the best antagonists can function well under the complete range of conditions favoring a pathogen. Otherwise, if conditions occur that only favor the pathogen, the antagonist would lose any advantage it might have and disease would develop.

The concept of the *r*-*K* continuum is often linked with concepts of succession, with *r*-selected species being considered as early colonists and *K*-selected species the climax species. Three species of antagonists were found attacking the cereal cyst nematode (*Heterodera avenae*) in suppressive soils. The obligate bacterial pathogen *Pasteuria penetrans* infected second-stage juveniles, the fungus *Nematophthora gynophila* infected developing females, and the facultative fungal pathogen *Verticillium chlamydosporium* infected eggs (Davies *et al.*, 1990). These antagonists were thus affecting different stages in the nematode life cycle and have been thought of as specializing at different stages in succession, as the nematodes developed. Knowledge of this specialization would clearly be essential when considering these antagonists for control purposes.

16.3.2 Studying antagonists and plant pathogens

Molecular biology has become an increasingly important tool for microbial ecologists in recent years. For example, molecular techniques

provide marker genes (also called molecular markers), which allow microbiologists to follow the populations of a specific microbial strain in a natural habitat. Using molecular markers, plant pathologists have developed a far better understanding of the population dynamics of specific plant pathogens and interactions of plant pathogens with naturally occurring or introduced antagonists in soil and on plant surfaces, where biological control occurs. Some molecular markers, such as the gene encoding the Green Fluorescent Protein (obtained from a jellyfish), allow microbiologists to monitor individual microbial cells on plant surfaces. These markers are becoming increasingly important in evaluating the ecology of microorganisms in natural habitats.

Using molecular biology, plant pathologists have also been able to develop mutants of antagonists that differ from the parental strain in only one very specific way. Such mutants have become extremely important tools for identifying the mechanisms used by antagonists to suppress plant disease. For example, some antagonists produce antibiotics that are toxic to their target pathogens, inhibiting pathogen growth on culture media in a laboratory. Because microorganisms need sufficient nutrition to produce antibiotics, however, there were good reasons to question whether antibiotics were produced by antagonists in natural habitats in the quantities needed to suppress target pathogens. Using molecular biology, plant pathologists created mutants of antagonists that are deficient only in antibiotic production. If an antibiotic-producing antagonist suppresses plant disease whereas an antibiotic-deficient mutant does not suppress disease, then plant pathologists can conclude that antibiotic production is an important determinant of biological control in this pathogen/antagonist system.

16.4 | Interactions among microorganisms

Antagonists interact with pathogens in numerous ways. In some instances interactions are directly due to one microbe suppressing the activity of another. Their interactions are based on either (1) excluding a pathogen through competition for space and/or nutrients, (2) producing antibiotics to kill competitors, or (3) antagonists directly destroying pathogens by one microbe parasitizing another (Campbell, 1989). Interactions can also be indirect, as when the presence of one microbe induces resistance in the host plant toward the same or a different plant pathogen. Indirect effects are also possible when manipulation of the environment alters the microhabitat so that the species composition and abundance of the community of microorganisms changes, with the end result of decreased densities of plant pathogens. We will discuss each of these mechanisms of interaction separately, although it is thought that in nature several mechanisms are often used by the same species.

16.4.1 Resource competition

Competition occurs when two microbes need the same limiting resource and, once one microbe has gained access, that resource is not available to the other. Microbes generally are thought to compete for nutrients, in particular carbon and nitrogen. They are also frequently quite sensitive to environmental conditions so space that provides protection as well as conditions for growth can be limiting. Water is required by virtually all growing stages of microbes and competition can occur for microhabitats where water is available. In particular, little (usually $< 1\%$) of the surface of leaves is occupied by microbes because of the low levels of available water and nutrients on leaf surfaces. On leaf surfaces, there are few suitable or optimal locations for microbes to establish and grow and microbes compete for the few favorable sites that occur.

Roots provide a different situation because exudates that can be used by microbes can be produced along root surfaces. Microbes live along the surfaces of roots but still only a limited amount of space and nutrients is available in this defined habitat. The area around a root, or the rhizosphere, is critical for soil-borne pathogens as well as for their antagonists. The microbial dynamics around roots for limited space and nutrients can be intense. As roots grow, some microorganisms can spread with them and retain their activity. This is especially important because many pathogens infect at the root tip, which is not a static entity. Antagonists with this ability to spread as plants grow are often applied to seed or to plants at the soil surface or to upper soil levels. Those microbes whose populations persist over time, increasing to large populations in the rhizosphere, and spreading to lower portions of the root, such as the root tip, are called rhizosphere competent (Baker & Griffin, 1995).

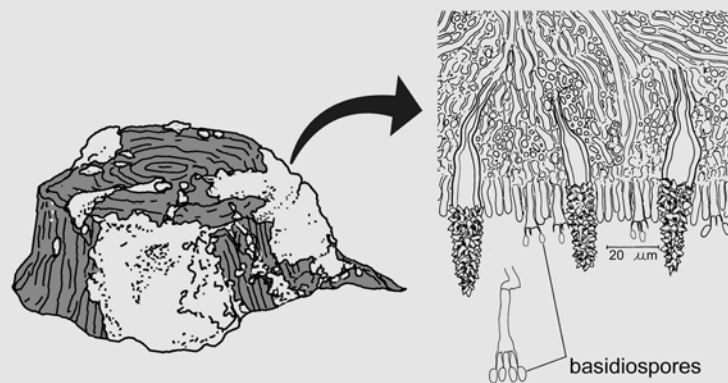
Competition between a pathogen and an antagonist has been used for biological control purposes at planned wound sites, such as when cuttings are made, during replanting, or when trees are cut and stumps need to be protected. In these cases, non-pathogenic microbes that are good competitors are applied directly after wounding. These antagonists colonize this excellent growth environment and, in doing so, restrict colonization by pathogenic microbes that would colonize but then cause disease. The first biological control agent commercialized to control plant pathogens uses this strategy to control spread of root pathogens in forest stands (Box 16.1).

Box 16.1 | Fungus stumps fungus

After forests are harvested, tree stumps remain. What we cannot see is that under the ground, as trees grew their roots had spread out and had grown together with roots of neighboring trees via root grafts before the trees were harvested. After a pine is cut, spores of the pathogenic fungus *Heterobasidion annosum* are soon deposited on the freshly cut stump by the wind and these spores then establish an

infection. This virulent pathogen does not stop in that stump, but grows into the root system and continues growing through root grafts and thus spreads to the root systems of nearby healthy trees. Disease caused by this fungus, called root and butt rot of conifers, is a problem in tree stands where only some of the trees are cut and others remain. The goal of this silvicultural practice called thinning is to promote growth of the trees that remain, so exposing them to pathogens due to thinning is certainly not the desired outcome.

The fungus *Phlebiopsis gigantea* (previously known as belonging to the genera *Phanerochaete*, *Peniophora*, and *Phlebia*) is a great competitor and colonizer of wounds but is not pathogenic to living trees. If this fungus is applied to tree stumps just after cutting them, it will spread through the stumps and roots and will become well established, occupying this resource before *H. annosum* arrives. Even if *H. annosum* is already in the stump but is not well established, *P. gigantea* can exclude or replace it. *P. gigantea* antagonizes *H. annosum* through a phenomenon known as hyphal interference; when hyphae of *P. gigantea* contact hyphae of *H. annosum*, the protoplasm within the *H. annosum* hyphal cells becomes disorganized and the cell membranes appear leaky.



Phlebiopsis gigantea growing on a tree stump. In some areas of the fungal growth, infective basidiospores are produced. (Close-up of fungus from Eriksson et al., 1981.)

P. gigantea is common in the environment but its natural levels are too low and sporadic to provide control of *H. annosum* naturally. *P. gigantea* began to be mass-produced for use against *H. annosum*, but developing a method for applying this fungus to cut stumps was a challenge. In time, several different methods for application were developed. Fungal spores can be applied as a powder or water suspension to a cut stump. A clever and faster alternative was mixing the spores in the lubricating oil of the chain saw used for cutting the tree and the fungus was then deposited in the wound as the tree was cut. *P. gigantea* for control of *H. annosum* was first commercialized in 1962 (Rishbeth, 1975), becoming the first product for biological control of a plant pathogen. Described in the literature in 1963, this system became the first operational use of biological control for control of a plant pathogen. This fungus is available today in England, Sweden, Norway, Switzerland, and Finland. It was available until 1995 in the USA at which time the US Environmental Protection Agency discontinued its availability until registration requirements were addressed and satisfied.

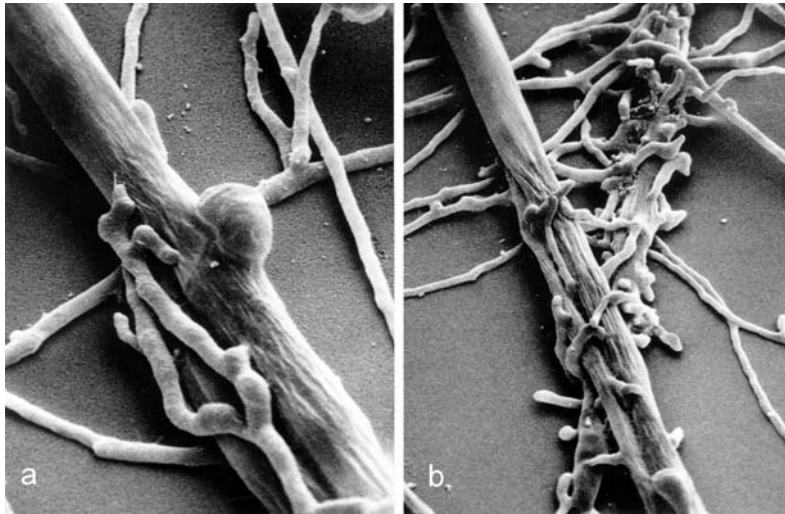


Fig. 16.1 Scanning electron micrographs illustrating mycoparasitism. a. The chromist *Pythium acanthicum* parasitizing hyphae of the basidiomycete *Corticium*. b. *Pythium acanthicum* parasitizing *Phycomyces blakeleeanus* hyphae, which appear collapsed. (Hoch & Fuller, 1977.)

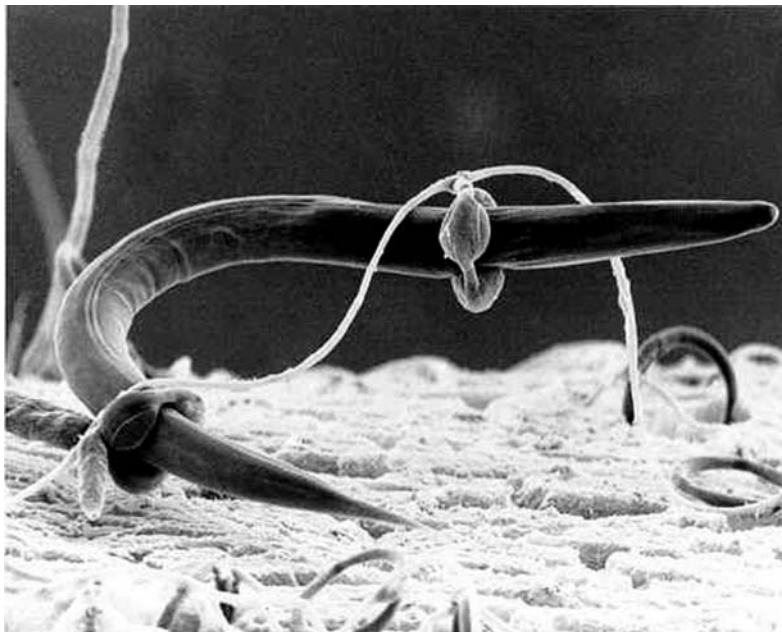
One form of nutrient competition involving access to iron is being investigated for iron-limited soils, such as arable soils on limestone rocks or other high-pH soils. In clay soils, lime can be added to improve the aggregate structure; this causes a high pH with the result that ferric iron is precipitated out as ferric hydroxide. In these instances, iron becomes unavailable to microorganisms. Some microorganisms produce compounds that bind iron, called siderophores, and these siderophores vary in their ability to bind iron. It has been postulated that if antagonists that produce siderophores colonize roots, the pathogens in that microhabitat could then be limited by iron. To demonstrate this, antagonists producing siderophores can be effective at controlling pathogens while mutants of these same species that do not produce siderophores cannot control pathogens. Although the use of siderophores for biological control is not yet available due to the complexity of these interactions, studies with siderophore-producing antagonists clearly demonstrate potential for control.

16.4.2 Parasitism

Some microbes directly attack other microbes and use them as sources of nutrients. Such parasitism requires direct contact between microbes; it is often referred to as hyperparasitism, or mycoparasitism when interactions involve a fungus. Mycoparasitism is most visually demonstrated by fungal parasites that penetrate through the tubular body form (hyphae) of plant pathogenic fungi for access to the hyphal contents (Fig. 16.1).

Perhaps the best-known examples of mycoparasites are species of the fungal genus *Trichoderma*, which are often used for suppression of diseases caused by pathogens occurring in the soil. Hyphae of *Trichoderma* species can penetrate through the walls of fungal resting structures and can also parasitize the tubular hyphae. They often wind themselves around the hyphae of their host and produce side

Fig. 16.2 A nematode captured by constricting rings of the predatory fungus *Arthrobotrys anchonia*. Ring cells swell and eventually constrict the body of the nematode, and then germinate to produce invasive hyphae that penetrate the living body of the nematode. The fungus subsequently grows through the host's body, digesting the contents. (Barron, 1979.)



branches that are seen to coil around individual hyphae and penetrate through host walls. Although the impact of *Trichoderma* species on their microbial hosts is obvious, the overall importance of mycoparasitism to biological control of plant disease has been questioned. *Trichoderma* species, like many antagonistic microbes, utilize several different strategies to suppress plant disease. The antagonist *Gliocladium virens*, which is closely related to *Trichoderma*, produces antibiotics as well as acting as a mycoparasite. When *G. virens* was altered to study its mode of action, a mutant that no longer formed coils around host hyphae still provided effective disease control, suggesting that the readily visible mycoparasitism is not required and is only one part of the antagonism caused by *G. virens*.

Some of the major antagonists affecting plant parasitic nematodes are fungi. These fungi produce spores that adhere to the nematode cuticle before infecting the nematode. Several fungal species effective against root-knot nematodes are endoparasites, growing within nematodes before killing them. In particular, *Verticillium chlamydosporium* shows promise because this endoparasite produces thick-walled resting spores for persistence in the soil, a valuable feature for an antagonist (Kerry, 2001). Most fascinating are those fungi whose hyphae grow into rings when nematodes are present, such as *Arthrobotrys anchonia*. When a nematode swims through a ring, the ring constricts around the nematode, trapping it (Fig. 16.2). The body of the nematode is subsequently invaded by the fungus and the nematode dies. These beneficial fungi naturally occur in soils and efforts to increase their efficacy in controlling nematodes are focused on making the soil environment more amenable to these nematode-attacking fungi (see Chapter 17).



Fig. 16.3 Example demonstrating antibiosis between two pathogen strains in a petri dish. Different strains of a pathogen have been inoculated at right and left and a potential antagonist has been inoculated at top and bottom. Note the wide inhibition zone caused by the diffusion of antibiotics from the antagonist. (Campbell, 1989.)

16.4.3 Antibiosis

The term antibiosis has its roots in the term antibiotics, which refers to organic substances produced by microorganisms that, even at low concentrations, are deleterious to the growth and metabolic activity of other microbes. Antibiosis therefore refers to the inhibition of one microorganism by an antibiotic produced by another microbe. The result of antibiosis is often death of microbial cells by endolysis and breakdown of the cell cytoplasm.

One advantage of using antibiosis for control is that antibiotics produced by an antagonist can diffuse in films of water or through moist soil so that actual physical contact between the antagonist and the pathogen does not have to occur. This is perhaps best seen when cultures of an antagonist grow on an agar plate near a susceptible microbe and an unoccupied zone of inhibition surrounds the colony of the antagonist: this zone is caused by diffusion of an antibiotic produced by the antagonist into the surrounding agar medium (Fig. 16.3). There is compelling evidence that certain antagonists produce antibiotics when they are colonizing the rhizosphere, seed surfaces, or plant wounds, and that the concentrations of antibiotics produced are adequate to suppress target plant pathogens. One example of antibiosis being harnessed very effectively to control a pathogen is biological control of the disease called crown gall which is caused by the bacterial pathogen *Agrobacterium tumefaciens* (Box 16.2).

Antibiosis has many advantages, so why not always use antagonists for biological control of plant pathogens that use antibiosis as a strategy? First, antibiosis seems to be more effective when nutrients are abundant or excessive in the microbe's microhabitat. Antagonists produce antibiotics only when nutrients are available and not all microbial habitats on plant surfaces or in soil have adequate nutrients for antibiotic production. Antibiotics are not normally produced by microbes in soil and generally do not persist for long in the environment. Therefore, microhabitats where antibiotics are present are limited in area and in time. In addition, antibiotics differ in the number

Box 16.2 | Biological control of crown gall

When rootstocks or wood of nursery stock are cut or grafted, an excellent location is provided for the bacterium *Agrobacterium tumefaciens* to invade. Once this bacterial species has entered a wound, it causes uncontrolled growth in that area of the plant stem, resulting in production of a gall, often at the crown of the plant where the stem enters the soil. Valuable perennial plants such as peach, plum, almond, and other fruit trees are susceptible, as well as vines and other herbaceous plants, making a total of 93 families of plants that can be affected by this gall-forming species of bacteria.



Crown gall occurring at the junction of an apple stem with the ground. (Photo courtesy of Cornell University, Dept. Plant Pathology.)

The virulence of *A. tumefaciens* is due to a plasmid, an extrachromosomal piece of DNA that can replicate independently of the bacterial chromosome and can be transferred between organisms. Strains of *A. tumefaciens* can gain or lose virulence based on presence or absence of the Tumor-inducing, or Ti-plasmid. When the bacterial cells enter a potential host plant, part of the plasmid is transferred to the host cell and is expressed to stimulate the uncontrolled plant growth creating the gall.

The bacterial antagonist *Agrobacterium radiobacter* strain K-84 is closely related to *A. tumefaciens*, but does not have the Ti-plasmid and thus does not cause crown gall. *A. radiobacter* K-84 can colonize surfaces of wounds on roots or stems of nursery stock and, if it is present at a wound or a site where *A. tumefaciens* could potentially infect, it will prevent the pathogen from becoming established and thus no galling occurs. *A. radiobacter* K-84 produces an antibiotic, agrocin 84, that is toxic

to many strains of *A. tumefaciens* (Kerr, 1980) and this antibiotic has an important role in biological control of crown gall.

A. radiobacter K-84 was recognized as a valuable control agent and was first developed commercially for control of crown gall in 1973. Cuttings, transplants, or root-pruned seedlings are dipped into a water-based suspension of *A. radiobacter* and then planted. *A. radiobacter* then colonizes any wounds and *A. tumefaciens*, which is common in the soil, cannot establish an infection, although *A. radiobacter* must be present at the wound first. Antibiosis can be very specific; the strain of *A. radiobacter* that has been commercially available since 1973, K-84, is not effective against strains of *A. tumefaciens* that attack grapes, pome fruit (e.g., apples), and some ornamentals, although it is very effective against *A. tumefaciens* attacking stone fruit (e.g., peaches and plums). Although the strain K-84 has been used very successfully for biological control, there have been reports of breakdown in its effectiveness. This has been attributed to transfer of some genetic material responsible for producing the antibiotic agrocin 84 from K-84 to the pathogen, thus making the pathogen immune to agrocin 84. However, it has been possible to splice out genetically the small region of the DNA in K-84 that allowed the transfer of the gene encoding agrocin. The new bacterial strain with this deletion, strain K1026, is now commercially available and is effective on plants such as roses and raspberries in addition to peaches and plums.

of microorganisms that they damage and some antibiotics have narrow specificities. Lastly, many antagonists do not produce antibiotics but still effectively suppress disease through other mechanisms.

16.5 | Indirect effects

In some cases, antagonists suppress disease indirectly by inducing resistance responses in the plant rather than by directly suppressing populations of a target plant pathogen.

16.5.1 Microbial infections inducing resistance

Although plants do not naturally produce antibodies, they have their own methods for building protection against microbial invaders. If certain plant species are inoculated with specific pathogens, this can lead to temporary or sometimes almost permanent immunization, or induced resistance, in the plant. While this defense response can help to protect against all pathogens, it is specifically important against plant pathogenic viruses, for which few other types of control are effective.

One type of induced resistance, called cross protection, has been used to manage diseases caused by viruses. There can be many strains of any one plant virus and these strains can vary dramatically in the severity of disease that they cause. The minor damage caused by mild strains of some viral pathogens can be tolerated easily by growers, and it has been found that those mild strains can be used as biological control agents. When a plant is inoculated with a mild strain of

some viral pathogens, the plant becomes protected from infection by more virulent strains of the same virus. Cross protection has shown promise in controlling viral diseases in tomatoes and is used widely in Hawaii for control of papaya ringspot. Citrus tristeza is a very serious disease affecting sensitive varieties of citrus, and is caused by a virus principally vectored by aphids. It is native to Africa but was introduced to South America some time in the 1920s and became widespread. Mild strains of the virus were found and these became the basis of a cross-protection program. Cross protection is somewhat like human vaccination; a bud from a tree infected with the mild strain of the virus is grafted onto a healthy tree and the mild virus spreads throughout the tree to protect it against infection by the virulent strains of the virus. Researchers found that citrus tristeza cross protection is not broken down through time. The first cross-protected citrus plants were distributed in Brazil in 1968. By 1980, eight million Pera sweet orange trees (*Citrus sinensis*) in Brazil were cross protected (Costa & Müller, 1980) and use of cross protection against citrus tristeza continues around the world today. Cross protection is not used on more types of plants because mild strains of appropriate viruses are not always available or effective. This strategy also is not appropriate for field crops where resistance would have to be induced in many individual plants that often will not be present the next year. In perennial crops, resistance due to cross protection can fade after a few years and, when it is active, cross protection sometimes can be unevenly distributed through larger plants such as trees.

Systemic acquired resistance (SAR), in which a plant infected with one pathogen is more resistant to another, is thought to show more promise than cross protection. SAR can be induced when a resistant stage of a plant species is inoculated with a pathogen. Later, when the plant matures to a susceptible state, it will be resistant to that same pathogen as well as to other pathogens. Bean and sugar beet plants inoculated with virus are subsequently more resistant to infection by certain obligate fungal pathogens such as rusts and powdery mildews, when compared with virus-free plants (Agrios, 1997). Young cucumber plants were inoculated with either a fungus, a bacterium, or a virus, and after a few days were resistant to 13 diseases caused by fungi, bacteria, and viruses. Interestingly, resistance must be induced in the plant prior to the onset of flowering or fruit production. SAR is a delayed but prolonged response; if SAR is induced in cucumber plants, after a few days they are protected from all pathogens for 4–6 weeks.

While the exact biochemical events responsible for SAR are still unknown, SAR is associated with at least nine families of genes with products directly exhibiting antimicrobial activity, for example β -1,3 glucanases, chitinases, or other antimicrobial proteins. The SAR genes induced in a plant can be specific to that plant species and to the agent (pathogen, saprophyte, or chemical) that induces the resistance response. In recent years, plant pathologists discovered that some non-pathogenic microorganisms can induce SAR. For example,

some rhizosphere-inhabiting microorganisms have been shown to induce SAR, which results in reduced severity of both root and foliar plant diseases. Different biochemical pathways may be involved in the SAR induced by these non-pathogenic rhizosphere microorganisms versus the SAR induced by pathogens. Use of SAR for plant protection has progressed away from use of living microorganisms to induce resistance after researchers found that resistance could be induced using naturally occurring compounds obtained from pathogens or even non-pathogens, such as salicylic acid.

Some non-pathogenic microbes are known to elicit induced systemic resistance (ISR) in plants. Presence of plant growth-promoting rhizobacteria (PGPR) in the soil has been associated with increased rates of plant growth and numerous PGPR strains, including the commercially available *Bacillus subtilis*, have also demonstrated biological control activity against numerous soil-borne pathogens. Researchers found that PGPR strains remaining on the roots induced resistance to foliar or systemic pathogens by activating the plant's defense system. Once activated, the induced systemic response is maintained for prolonged periods against multiple pathogens, even if populations of the inducing bacteria decline over time. This is an active area of study and ISR seems to hold great potential, especially in cases such as insect-transmitted plant pathogens that are often difficult or impossible to control with pesticides.

16.5.2 Mycorrhizae

Many plants maintain symbiotic relationships with certain kinds of fungi associated with their roots. These relations are generally thought to be mutualistic, with both of the partners benefitting from the relationship. Fungal root associates, or mycorrhizae, either grow extracellularly (ectomycorrhizae) or, more commonly, intracellularly (endomycorrhizae). Mycorrhizae obtain organic nutrients from the plant and they help the plant by enhancing nutrient uptake and water transport. Mycorrhizae gain a sheltered place to live with abundant nutrients and the plant increases in growth and yield. This relates to biological control because sometimes plants with mycorrhizae have also been found to be protected against some soil-borne pathogens. For example, pine seedlings with mycorrhizae were protected from the pathogenic chromist *Phytophthora cinnamomi* and cotton with mycorrhizae was protected against the fungus *Verticillium dahliae* that causes wilt, as well as from root-knot nematodes. The exact mechanisms for protection when mycorrhizae are present have not been elucidated.

Some mycorrhizae are available commercially for application to promote plant health. However, these fungi are difficult to work with, mass-produce, and apply and, while they are used to promote plant health, they are not yet specifically used for protection against or control of plant pathogens.

FURTHER READING

- Agrawal, A. A., Tuzun, S. & Bent, E. (eds). *Induced Plant Defenses Against Pathogens and Herbivores: Biochemistry, Ecology and Agriculture*. St. Paul, MN: American Phytopathological Society, 1999.
- Andrews, J. H. *Comparative Ecology of Microorganisms and Macroorganisms*. New York: Springer-Verlag, 1991.
- Barron, G. L. *The Nematode-destroying Fungi*. Guelph, Canada: Canadian Biological Publications, 1977.
- Beattie, G. A. & Lindow, S. E. The secret life of foliar bacterial pathogens on leaves. *Annual Review of Phytopathology*, 33 (1995), 145–172.
- Bellows, T. S. 1999. Foliar, flower, and fruit pathogens. In *Handbook of Biological Control*, ed. T. S. Bellows & T. W. Fisher, pp. 841–852. San Diego: Academic Press.
- Butt, T. M., Jackson, C. & Magan, N. (eds). *Fungi as BioControl Agents: Progress, Problems and Pitfalls*. Wallingford, UK: CABI publishing, 2001.
- Campbell, R. *Biological Control of Microbial Plant Pathogens*. Cambridge: Cambridge University Press, 1989.
- Jeffries, P. & Young, T. W. K. *Interfungal Parasitic Relationships*. Wallingford, UK: CAB International, 1994.

Microbial antagonists combating plant pathogens and plant parasitic nematodes

In the field of plant pathology, the focus for biological control is on suppressing plant disease, much more than on thinking of biological control as controlling specific individual organisms that cause disease. It follows that emphasis is placed on use of antagonists to make certain that plants are not injured, while entomologists working in biological control often place more emphasis on impacts of natural enemies on densities of specific pests.

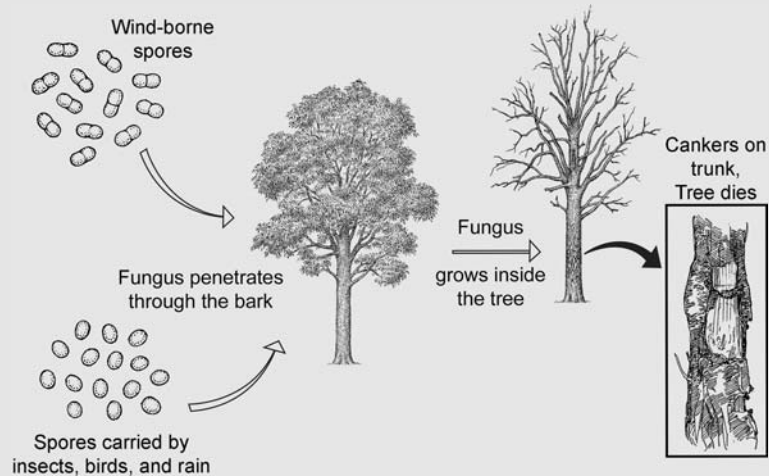
17.1 Finding antagonists

When antagonists are present in the correct microhabitat and are active, plant disease does not occur. However, lack of disease does not especially prove that antagonists are present because while the pathogen may be absent conditions might not be correct for the pathogen to be active so its presence would go undetected. Even if disease is occurring, antagonists might be present yet inactive. How are antagonists of plant pathogens found? Typically, they are isolated from the same habitats where their target pathogens live – from plant tissues or from the soil. Sometimes in a field of diseased plants, a few plants are very obviously healthy. These healthy plants might have escaped disease due to the presence of naturally occurring antagonists that suppress the pathogen affecting neighboring plants. Microbes isolated from the healthy plants have the promise of being antagonists. Antagonists occurring in the soil have been identified in much the same way as those isolated from healthy plants. When diseases that might be expected never occur in specific regions, antagonists of plant pathogens have been found in the soils. In these instances, soils have been called suppressive and suppressive soils have been one focus of research in biological control of plant disease (see below).

In some cases, the same species of microbe as the pathogen has been isolated from a healthy plant, but it is a non-virulent strain. In fact, many microorganisms have strains within the same species that vary in virulence (the relative ability to cause disease), from highly aggressive strains to strains living within hosts but not having

Box 17.1 | Virus weakens fungus attacking chestnut trees

The American chestnut (*Castanea dentata*) used to be a common and valuable tree species in many forests of the northeastern USA. In the early 1900s a fungal pathogen, *Cryphonectria parasitica*, was first found in New York City, attacking American chestnut trees. This virulent fungus causes wounds called cankers on tree trunks and, after several years, kills infected trees by girdling them. *C. parasitica* swept across North America and after it had spread, the once-common American chestnut trees were rare due to this so-called chestnut blight.



Chestnut blight. Windborne sexual spores and asexual spores transported by insects, birds, and rain land on the bark of a chestnut tree and penetrate, with fungal growth subsequently spreading within the tree. Cankers, delimited dead areas on the trunk, eventually kill infected trees by blocking the exchange of water and nutrients between leaves and roots. (Newhouse, 1990; Canker drawing courtesy of F. Paillet.)

Just before World War II, for the first time the native chestnut trees in Europe began to show signs of infection and began dying rapidly. However, about 20 years later, it was noticed that some sucker sprouts from infected trees were surviving well in Italy. Researchers learned that although the same fungus occurred in these sprouts, it was not as virulent. Detailed studies went on to demonstrate that this fungal strain with reduced virulence (a hypovirulent strain) was morphologically distinct, having white instead of orange pigmentation, reduced spore production, and slower growth. Furthermore, when the hypovirulent strain grew in the same dishes as the virulent strain, if the hyphae grew together, the virulent strain would become hypovirulent. Research demonstrated that DNA typical of viruses resided within the hypovirulent strain and this DNA could be transferred when cells of virulent and hypovirulent strains grew together; thus transferring the trait for weaker virulence. Wonderfully, this transfer also occurred within infected trees. If a tree was infected with the virulent strain and then inoculated with the hypovirulent strain, although the fungus remained present, it no longer harmed the tree.

Further research demonstrated that this system is more complex than originally thought; several viruses are known to affect the virulence of this fungus,

components of viruses are present but not viral particles themselves, a cytoplasmic genetic factor also occurs, and the hypovirulent strains of *C. parasitica* found in North America differ from those in Europe.

In Europe, where chestnut products are popular as food, fungal epidemics in chestnut trees resulted in decreased availability of chestnuts for many years. Hypovirulence was not widespread so, after its discovery, the hypovirulent strain was isolated and was applied widely throughout Europe to suppress the disease. After a single introduction on a tree, control persists because the hypovirulence is established throughout the tree. European chestnut farmers today can purchase tubes of hypovirulence paste and apply it around cankers on trees that display symptoms of the virulent strain of *C. parasitica* (Fulbright, 1999).

Will blight end the chestnut?
The farmers rather guess not
It keeps smoldering at the roots
And sending up new shoots
Till another parasite
Shall come to end the blight.
Robert Frost

a negative impact. This is the case with a fungal pathogen infecting chestnut trees that can occur as either virulent or non-virulent strains (Box 17.1).

Finding an antagonist or a non-virulent strain of a pathogen can be the easier step compared with fully understanding the mechanisms used by an antagonist to prevent disease. In some instances, the mechanism of antagonism by a natural enemy being used for biological control is still not understood in detail. Of course, parasites that visibly attack microbes employ the most obvious type of antagonism but we have learned that this obvious activity is not always the only mechanism used by an antagonist against a plant pathogen (see below).

17.2 | Types of antagonists

Antagonists are taxonomically diverse, as are the pathogens they are used against. Fungi and bacteria are principally used for biological control against fungal and bacterial plant pathogens. Fungi can be used to control either fungi or bacteria and bacteria can be used to control either fungi or bacteria. No one mechanism of action is used only by bacteria or only by fungi. Aside from some of the species of microbes that are obligate parasites and are thus more specialized, many antagonists of plant pathogens occur naturally in the environment and can be ubiquitous and common members of the microbial community because their occurrence is often not dependent on the presence of a pathogen. Viruses can act as antagonists too but their mode of action is completely different, creating induced plant defense responses.

17.2.1 Fungal antagonists

Many of the fungal pathogens used as antagonists belong to the Phylum Ascomycota, or the sac fungi. This group includes many species that look like molds. The sexual spores of the Ascomycota are produced in groups of eight within small sacs, or asci. A complete fungal life cycle usually includes both sexual and asexual stages but the taxonomy of fungi is based on the sexual stages. Many fungal life cycles are complex and this group of fungi is hardly the exception. It is characteristic for many members of the Ascomycota usually to exist in the asexual form in which the spores (conidia) are produced on hyphae or are borne on fruiting structures. This situation can lead to confusion regarding names and affinities of fungal species that rarely, or virtually never, occur as their sexual stage. When a sexual stage of one of these fungi cannot be found, mycologists have devised a method of using a somewhat temporary name for the asexual stage; these fungi are grouped in the Deuteromycetes, also called Fungi Imperfecti (imperfect because they rarely, if ever, produce the sexual stage of that species).

Although many of the fungal species used for biological control belong to the Fungi Imperfecti, antagonists from other groups of fungi are also important. A fungus growing as single cells, the yeast *Candida oleophila*, is used to control pathogens attacking fruits and vegetables after they have been harvested. The fungus applied to stumps of conifers, *P. gigantea* (Box 16.1), belongs to the group of fungi that includes most mushrooms, the Basidiomycota.

Those natural enemy species providing control through competition are usually not specifically associated with one species of pathogen; their association is based on using a microhabitat that is also preferred by the pathogen. Many antagonists thus occur commonly in nature. Different species are important for controlling pathogens attacking virtually all of the different locations on plants. Perhaps the greatest use for antagonistic fungi has been in suppression of soil-borne plant pathogens. However, fungal antagonists have also been developed for control of pathogens attacking tree wounds, foliage, and harvested produce.

17.2.2 Chromist antagonists

Some antagonists are water molds, a group once called the Oomycetes and thought to be fungi. However, these species are now classified within the Chromista. For example, the chromist *Pythium oligandrum* is an antagonist that suppresses diseases caused by phytopathogenic fungi and chromists through competitive interactions.

17.2.3 Bacterial antagonists

Many of the bacterial species utilized as antagonists are active against soil-borne plant pathogens. Members of the family Pseudomonadaceae (e.g., *Pseudomonas fluorescens*) and Actinomycetes (e.g., *Streptomyces griseoviridis*) often use combinations of competition and

antibiosis to prevail in the complex soil environment. *Pseudomonas* species are also used for suppression of diseases of aerial plant parts as well as soil-borne diseases.

Some of the bacterial antagonists of plant pathogens belong to the Family Bacillaceae, the same family as the insect pathogen *Bacillus thuringiensis*. These are, of course, spore-forming bacteria and several are known to produce antibiotics, just as Bt produces a secondary metabolite toxic to insects. These spore-forming species have the ability to persist in the environment and this characteristic prolongs their presence after application. Members of the Bacillaceae are applied against foliar as well as soil-borne pathogens.

17.3 Strategies for using antagonists to control plant pathogens

Methods for use of these different types of microorganisms for control differ by the type of plant and pathogen. In stark contrast with biological control of invertebrates or weeds, classical biological control has been applied against plant pathogens in only a few instances. Biological control of chestnut blight is one instance of classical biological control (Box 17.1). In a second instance, two parasitic fungi (*Dicyma pulvinata* and *Cylindrosporium concentricum*) were introduced against a fungal pathogen causing black crust on the foliage of rubber, *Phyllachora huberi*, in the Amazon basin of Brazil and long-term control was documented (Sutton & Peng, 1993). These antagonists are obligate pathogens living as hyperparasites and their increase is dependent on the presence of the plant pathogen. After the fungal antagonists were first released, the subsequent effects on the pathogen were cyclical. The fungal parasites require the pathogen for reproduction so when the parasites kill the pathogen, many spores are produced that then proceed to protect the next set of leaves. However, on this second set of leaves, few spores were produced because there was so little disease and the third set of leaves were therefore not well protected.

Perhaps classical biological control of plant pathogens has not been used more frequently because, in many instances, the major requirements of typical classical biological control agents are not met. Most importantly, a major requirement for classical biological control is that the natural enemy species does not already occur in the area where it would be released. Studies based on molecular techniques have suggested that generalist, soil-dwelling species of microbes are widely dispersed (Felske & Akkermans, 1998), perhaps occurring worldwide. Many antagonistic interactions among microbes are based on competition for limiting resources and not only on host-specific interactions, so their occurrence is not tied to presence of a host. Classical biological control using obligate microbial pathogens with narrow host ranges is appropriate for controlling some weeds and insects with localized distributions. However, it seems that generalist antagonists of plant pathogens on crops grown around the world

are probably already worldwide in distribution. Therefore, there is no need to introduce them to new areas because they already occur there. For biological control of plant pathogens, the major ways that these generalist antagonists are released is through augmentation of natural enemy populations, and in specific systems antagonists are conserved or enhanced. However, augmentation, rather than conservation, is the most common approach for biological control of plant disease.

17.3.1 Augmentation: inundative versus inoculative releases

The principal way that antagonists of plant pathogens are used is by releasing them either where the plant pathogen already occurs or, prophylactically, on the plant surfaces, where they can protect the plant from infection by the pathogen. While all antagonists have been called biopesticides, those applied prophylactically are often called bioprotectants instead. Some plant pathologists have called all of these mass applications of antagonists “introductions” but this general term could be used in reference to several strategies, including classical biological control, or inoculative or inundative releases. So what do we call releases of antagonists against plant pathogens? Using our definitions for release strategies, an inundative release would occur when control is achieved exclusively by the organisms themselves that have been released (see Chapter 4). Thus, for an inundative release of insect natural enemies to succeed, large numbers of natural enemies are usually released. In contrast, most releases of microbial antagonists fit the definition of inoculative releases. Microorganisms increase so quickly that it is not the released organisms that are effective against the host but rather the generations of microbes produced after release that increase to colonize the complete habitat, sometimes producing antibiotics as they increase. We have defined inoculative releases as releases of natural enemies that need to multiply to be effective and that will thus control the pest for a more extended period than an inundative release. Antagonists that are applied in large amounts either as protectants to prevent infection by a pathogen or as biopesticides to control pre-existing pathogens must grow to colonize the sites where they will be active; in this way both types of applications (applied preventively or after disease is present) fit our definition of inoculative release. While the exact length of a so-called “extended period” of control can be subjective, many antagonists can protect against or inhibit plant pathogens for longer than an immediate effect. Therefore, in the vast majority of cases, releases of antagonists to control plant pathogens are inoculative releases; antagonists that are released inoculate the area to be protected, much as aliquots of microbes are used to inoculate media, and these microbes then increase and spread throughout the area necessary for colonization.

Antagonists of plant pathogens first began to be developed for augmentative release in 1962, when *Phlebiopsis gigantea* first became

available for suppression of root and butt rot of conifers (see Box 16.1), and the number of products has proliferated since then. Early products were certainly those species and strains of species that were easy to grow in culture and that seemed effective during laboratory studies. However, applications in the field did not consistently provide control. Scientists realized that a better understanding of activity in the field, especially within the soil, was necessary to produce effective biological control agents (Deacon, 1991). For example, the microbes released needed to be able to survive in the highly competitive root zone and laboratory studies did not always predict activity in the field. Therefore, detailed studies of persistence in this area were necessary, ultimately yielding improved products.

Numerous commercial products are produced worldwide for inoculative releases. In 2000, over 80 products based on approximately 29 species of microbes having activity against plant pathogens were near to production or were on the market (Whipps & Davies, 2000). Of these, 50 were fungal products, dominated by *Trichoderma* and *Gliocladium*, while 30 were bacterial, dominated by *Pseudomonas/Burkholderia* and *Bacillus*, and there was one product based on a virus. A sample of some of the commonly used antagonists is presented in Table 17.1. Products affect different types of plant pathogens on different plant parts using different mechanisms of action, although the antagonists for control of diseases caused by seed- or root-infecting pathogens predominate.

Antagonists that affect few plant pathogen species are generally those that act principally as obligate parasites while antagonists that employ numerous modes of antagonism, sometimes including parasitism, often have broader host ranges. Antagonists that suppress a number of diseases are more likely to be commercialized than those antagonists that suppress only one disease, because of the expanded market size offered by a broader spectrum of activity. Thus, many commercial biological control agents suppress more than one disease, due to their production of broad-spectrum antibiotics or their superior abilities to out-compete numerous pathogens for limited resources on plant surfaces. Perhaps of more importance to their development for control, those antagonists with broad activity are usually the easiest to mass-produce so these are the species that have been simplest to develop into commercial products. In addition, species producing long-lived survival structures, such as chlamydospores, for example *Trichoderma*, or endospores, for example *Bacillus*, are preferred for commercialization (Whipps & Davies, 2000) because these species can persist in a commercial formulation throughout production and storage, thereby ensuring that a viable product is made available to the consumer.

Because these are microbes, registration requirements can pose a serious burden before commercialization. However, numerous products are marketed as promoting plant health and can thus bypass the extensive toxicity testing required to register a microbial biological control agent in the USA (see Chapter 4). In some other countries,

Table 17.1 Some of the antagonists of plant pathogens available as biopesticides in 2001

Antagonist	Plant part	Disease and pathogen	Mode of action ¹
<i>Bacteria</i>			
<i>Agrobacterium radiobacter</i>	Woody stems and roots	<i>Agrobacterium tumefaciens</i> (causes crown gall)	Competition and antibiosis
<i>Bacillus subtilis</i>	Seeds Foliage	Damping off <i>Botrytis</i> , mildews and other fungi and bacteria	Competition Prevents germination, disrupts growth
<i>Pseudomonas fluorescens</i>	Seeds, roots	Damping off	Competition and antibiosis
	Flowers	<i>Erwinia amylovora</i> (causes fire blight)	Competition and antibiosis
	Mushrooms	<i>Pseudomonas tolaasi</i>	Competition and antibiosis
<i>Pseudomonas syringae</i>	Harvested fruit and vegetables	Fungal pathogens of stored products	Mechanism unknown
<i>Streptomyces griseoviridis</i>	Seeds, roots	Seed and soil-borne fungi	Competition and antibiosis
<i>Fungi</i>			
<i>Ampelomyces quisqualis</i>	Foliage	Powdery mildew	Hyperparasite
<i>Candida oleophila</i>	Harvested fruit	Fungi attacking stored fruit	Competition
<i>Coniothyrium minitans</i>	Roots	<i>Sclerotinia</i>	Parasite of long-lived sclerotia in soil
<i>Trichoderma harzianum</i>	Roots, seeds, foliage	Soil and foliar fungal pathogens	Parasitism, competition, induced resistance
<i>Chromista</i>			
<i>Pythium oligandrum</i>	Roots	Soil-borne fungal pathogens	Competition

¹ Mode of action not always completely understood.

however, registration is less of a hurdle and products are more readily available. In China, for example, between 1985 and 1993, “yield increasing bacteria,” largely based on *Bacillus* species, were applied to a cumulative area of 40 million hectares on over 50 different crops (Tang, 1994).

As with other types of biological control, antagonists are effective when plant pathogen populations are low and their use is not intended for controlling pathogens during disease epidemics. Of course, many of these antagonists are used to protect plants (as bio-protectants) so that pathogens do not even become established, a type of strategy not used for microbes used in biological control of

arthropods or weeds. The antagonists developed for inoculative release will be discussed below, based on the areas of the plant where they are effective.

Seeds and roots

The most common diseases targeted using biological control agents are soil-borne diseases. Historically, soil-borne pathogens have often provided the greater challenges for control compared with foliar pathogens and there are limited options for control. Both fungal and bacterial antagonists are sold for control of soil-borne pathogens and they usually compete with plant pathogens as well as using other mechanisms of action. While this all sounds simple, the soil is a hostile and very diverse environment and initial attempts to use biological control agents in this arena met with erratic performance. Costs of products were high because abundant amounts of materials were needed for application. Plant pathologists, without good alternative controls, were undaunted and proceeded to improve biological control methods. They found that pathogens affecting the greenhouse and nursery markets were often a better target than pathogens affecting field crops due to the controlled environmental conditions and the high value of the crops.

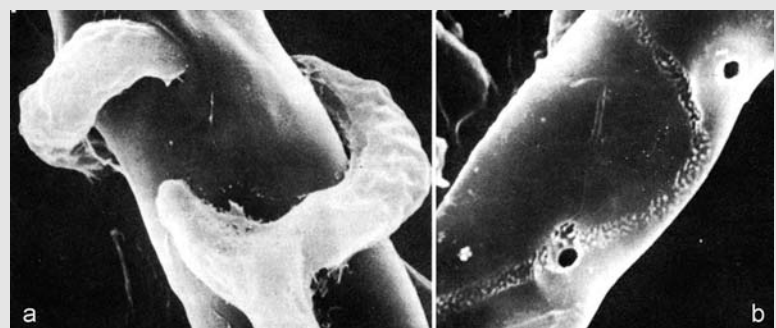
A pathogen well-established in a plant can be very difficult to control using any method. A clever change in designing biological controls was to apply natural enemies before disease organisms were present, for example application to seeds before planting. With this type of application, the seed surface is colonized by the antagonist and when the plant begins to grow, it is protected against pathogens that might kill sensitive seedlings, a general disease condition called damping off. Damping off, caused by fungi such as *Rhizoctonia* and chromists such as *Pythium* or *Phytophthora* spp., is a very serious disease in horticulture, agriculture, and forestry and can lead to almost total mortality in a planting. Many of the antagonists that control pathogens causing damping off are excellent competitors and rapidly colonize new locations after being introduced. The goal of such releases has been called pre-emptive exclusion of pathogens. This same idea has been used to protect cuttings and bulbs when they are transplanted by treating them with liquid suspensions of antagonists directly before planting. Alternatively, the biological control agents can be mixed directly into the soil or seed furrow before planting.

Similarly, control of root diseases is most successful if biological control agents are applied so that they are established in the rhizosphere before roots are colonized by a pathogen; roots that support established populations of a pathogen before an antagonist is applied are much more difficult to protect. Root tips are especially susceptible to infection by many soil-borne fungal pathogens; the susceptible root tips are constantly growing and moving through the soil where they encounter new pathogens. One strategy is to treat the entire rooting medium with an antagonist before planting. When applied to

furrows or to seed, rhizosphere-competent antagonists can colonize the root surface and spread along the root as it grows to offer protection from soil-borne pathogens. Some fluorescent pseudomonad bacteria that are antagonists are rhizosphere competent and will spread with growing roots. although, if applied to seed or the soil surface, these same bacteria require percolating water to move below 3 cm depth in the soil; therefore, in this case the method of application is critical for effective use of these antagonists. Many fungal antagonists are not rhizosphere competent although some *Trichoderma* are exceptions to this generality (Baker & Griffin, 1995). Some strains of the fungal species *Trichoderma harzianum* will spread to colonize growing roots where they employ a variety of methods for attacking or out-competing plant pathogens (Box 17.2). Several products based on *Trichoderma* species are available worldwide for suppression of numerous plant diseases.

Box 17.2 | The diversity of *Trichoderma*

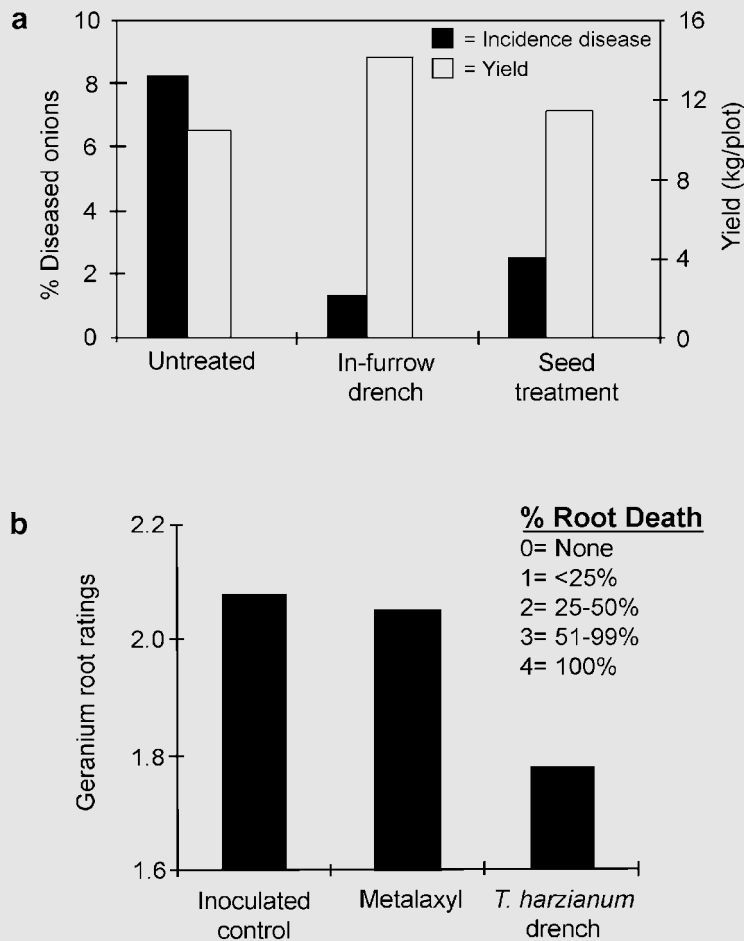
Perhaps the most unique antagonist that has been commercially developed is *Trichoderma harzianum* (Harman, 2000). This pathogen has defied the dogmas and myths associated with many antagonists developed for biological control. Through years of study, Gary Harman and colleagues have found that the activities of *T. harzianum* are diverse. It was once assumed to be effective predominantly as a mycoparasite. However, by 1997 and 1998, *T. harzianum* was found also to use antibiosis, to compete for nutrients and space, and to inactivate enzymes produced by pathogens. By 2002, research results suggested that this pathogen also has direct effects on the plants themselves; when *T. harzianum* is present, plants are more tolerant to stress because root development is enhanced and more nutrients are taken up.



a. Hypha of *Trichoderma hamatum* coiling around and penetrating a hypha of the plant pathogen *Rhizoctonia solani*. Partial degradation of the host cell wall can be seen. b. Hypha of the plant pathogen *Sclerotium rolsii* from which a coiled hypha of *Trichoderma harzianum* was removed, showing a digested area with holes where the antagonist penetrated. (Elad et al., 1983.)

T. harzianum was the first antagonist developed as a seed treatment for controlling fungi causing damping off of seedlings. However, its uses diversified with

improvements to the fungus and as understanding of this fungus increased. Several strains of *T. harzianum* are available commercially. However, one was specifically improved to strengthen certain characteristics; cells of two strains of *T. harzianum* that were each good biological control agents were fused together to create this new strain. The first was a strain that had been isolated from a suppressive soil and was good at surviving in the root zone. The second was a strain that was better at competing under iron-limiting soil conditions. From this union, a strain (T-22) that was optimally competitive and also able to survive well in the soil was used for development as a product for seed treatment.



Disease suppression by *Trichoderma harzianum* strain T-22. Asterisks indicate where differences were statistically significant. a. Incidence of onion basal rot caused by the fungal pathogen *Fusarium* and yield after treatments with *T. harzianum*, compared with untreated controls. b. Comparing damage by the chromists *Pythium* spp. on geraniums treated with the fungicide metalaxyl or *T. harzianum* or pathogen-inoculated controls. (Harman, 2000.)

Use of *T. harzianum* expanded when it was applied to soil as granules or as a drench to control fungi attacking roots and crowns of numerous crop plants, plants in nurseries, and golf course turf. This pathogen has also shown promise during

application with ozone for soil fumigation and as a spray to control fruit and foliar diseases. In one innovative application, researchers have studied whether bees can pick up spores of *T. harzianum* when exiting their hives and unwittingly deposit these spores on strawberry or other flowers; diseases of flowers like strawberry are often very difficult to control with fungicides and an effective alternative control method is needed. For some applications, other species of *Trichoderma* are added to expand efficacy, for example *T. harzianum* plus other species of *Trichoderma* are sold together for treating tree wounds or for protection of fruit after harvest. Thus, numerous products with *T. harzianum* are now available commercially.

As with many biological control agents, *T. harzianum* has limitations in the types of control situations where it can be effective. Use of this fungus should be strictly preventative because it cannot control existing diseases, especially under high disease pressure. As is typical of many agents principally used for augmentative releases, *T. harzianum* does not persist well in the environment in the absence of pathogens. However, it will persist to some extent if the roots of host plants are present, even if plant pathogens are not present, because some of the effectiveness of *Trichoderma* comes from being an effective competitor for space and nutrients and it does not always require a living pathogen for survival.

Stems and crowns

Plant stems vary in size and complexity from stems of annuals to tree trunks. As with transplanted cuttings, an open wound in a stem or crown provides an excellent location for entry and establishment of a pathogen. The antagonist products that have been most successful for the longest time are those applied at wound sites on trunks or stems to protect against pathogen invasion, as with the fungus *Phlebiopsis gigantea* colonizing pine and spruce stumps (Box 16.1) and the bacterium *A. radiobacter* protecting against crown gall (Box 16.2).

Post-harvest fruit

Both fungal and bacterial antagonists have also been developed to protect fruit against decay after harvest and during storage. Yeasts are good at colonizing plant surfaces under adverse environmental conditions and several yeast-based products have been developed (Fig. 17.1). Post-harvest pathogens are usually weak pathogens that need wounds on fruit to gain access. For suppression of post-harvest disease, fruit are often coated with antagonists before harvest to protect wounds that typically accompany harvest and post-harvest handling from infection. Alternatively, application can occur after harvest by dipping fruit into solutions containing the antagonist.

Foliage

Perhaps least well developed for biological control are the natural enemies combating foliar pathogens, possibly because this is the most common location where fungicides are applied so there is the least incentive to provide alternate controls. Some plant pathogens (e.g., *Botrytis*) that attack foliage are necrotrophic, with the ability to increase in dead plant tissues. In contrast, the commonly occurring



Fig. 17.1 Antagonistic yeast cells surrounding a spore of *Botrytis cinerea*, an important fungal pathogen causing post-harvest decay of fruits and vegetables. (Photo courtesy of Y. Egal and E. Fischer; Albajes et al., 1999.)

powdery and downy mildews attacking foliage require living hosts; while they seldom kill hosts, they can cause substantial decreases in growth and yield. Fungal antagonists can be applied to prevent damage by each of these types of pathogen. At least two of these antagonists, *Ampelomyces quisqualis* and *Pseudomyza flocculosa*, require high humidities after application for spore germination.

Bacterial diseases affecting aerial plant parts, such as fire blight (caused by *Erwinia amylovora*), can be extremely destructive. There is a very real lack of chemicals to treat these diseases and development of biological control against foliar bacterial pathogens is critically needed.

17.4 Conservation/environmental manipulation

Some plant pathologists have argued that, because augmentative releases are based on only one strain of one species of antagonist, they fail to take advantage of the great diversity of antagonists in the environment. Conservation strategies, also commonly considered as cultural practices in plant pathology, take advantage of the broad diversity present among naturally occurring antagonists in soil or on plant surfaces.

The environment is full of antagonists that could potentially inhibit activity of plant pathogens but to take advantage of their activity, conditions must be adjusted to favor persistence and increase of antagonists. Crop management strategies to conserve and enhance antagonists have actually been used for a long time. An extremely important disease management strategy worldwide, crop rotation, relies on the activity of antagonistic microorganisms in the soil (Mathre et al., 1999). Quite simply, when a crop is rotated every second or third year, the resident community of antagonists has a chance to lower the

inoculum levels of root- and foliage-infecting microbes while that crop is absent. At the turn of the century, researchers found that by adding compost, barnyard manure, and green manure to the soil, many soil-borne diseases could be suppressed. Since then, plant pathologists have learned that the addition of these amendments to soil enhances microbial activity, which is considered largely responsible for the disease suppression. Soil amendments can be especially useful in systems where crop rotation is not practical.

17.4.1 Disease suppressive soils

Plant pathologists have long known that when specific crops are grown in certain areas, disease problems that regularly occur elsewhere are not seen. In particular, this effect has been recognized for soil-borne diseases. Suppression of the plant pathogen is frequently due to numerous endemic microorganisms. Soils displaying this phenomenon of disease inhibition are said to be suppressive soils. In contrast, soils where a disease occurs are called conducive (non-suppressive) soils.

Agricultural soils suppressive to plant pathogens are known from around the world. There are two major types of suppressive soils. General suppression can occur due to the total microbial biomass in the soil and this effect cannot be transferred to other soils. Specific suppression is due to the effects of individual or specific groups of microorganisms. If this latter type of soil is added to sterile soil, the previously sterile soil will become suppressive and this activity is then said to be transferable. Generally, sterilization of suppressive soils often destroys this activity, demonstrating the biotic origin of the suppression. For specific suppression to develop in a soil, a specific pathogen needs to be present for some time, thus allowing the antagonists in the soil to increase.

The oldest known example of a suppressive soil is from the Chateaufrenard region in southern France. Melon plants are frequently susceptible to the soil-dwelling fungal pathogen *Fusarium oxysporum* var. *melonis* but, although the pathogen is present in this area, it does not cause the disease problems commonly found elsewhere. Saprophytic growth of the pathogen is suppressed along with germination of the persistent soil-borne stage, the chlamydospores. While some other varieties of *Fusarium* are suppressed in this area, other soil-dwelling pathogens are not affected. Many years of research were necessary to pinpoint the agents in the soil responsible for suppression. A non-pathogenic strain of *Fusarium oxysporum* and fluorescent pseudomonads (i.e., species of *Pseudomonas*) are thought to limit the pathogen through competition for nutrients and induced systemic resistance in plants. In fact, if organic matter is added to the suppressive soil so that nutrients are plentiful, suppression of the pathogen ends and *F. oxysporum* var. *melonis* once more becomes a problem. Interestingly, the non-pathogenic strain of *Fusarium oxysporum* seems to remain quite stable in its activity, which is consistent with the long-standing occurrence of these suppressive soils.

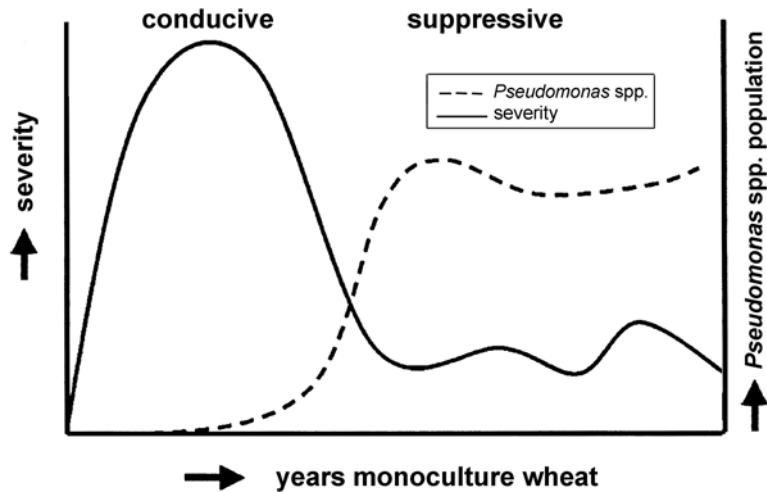


Fig. 17.2 Hypothetical model of the influence of a soil-dwelling fluorescent species of the bacterium *Pseudomonas* on take-all of wheat caused by the fungus *Gaeumannomyces graminis* var. *tritici*. With monoculture of a take-all susceptible host over several years, after one or more disease outbreaks, populations of these bacteria increase to densities over a threshold and soils that were conducive to growth of this pathogen become suppressive and take-all is controlled. (Weller *et al.*, 2002.)

Suppressive soils have been described for at least 15 more soil-borne pathogens (Weller *et al.*, 2002). Another excellent example of a suppressive soil involves a disease named “take-all,” caused by the fungal pathogen *Gaeumannomyces graminis* var. *tritici* that attacks the roots of wheat and occurs worldwide. Take-all can be managed by crop rotation, but this approach is not always practical economically. However, this is one example where monoculture without crop rotation has been shown to be beneficial for pest control. If wheat is grown continuously year after year, the severity of take-all increases for 4 to 6 years, but then begins to decline, a phenomenon called “take-all decline.” Take-all decline is attributed to suppressive soils that are built up during continuous wheat monoculture. Although numerous antagonists may contribute to take-all decline, one group of antagonists stand out; take-all decline occurs when certain antibiotic-producing strains of the fluorescent bacteria *Pseudomonas* spp. increase in soils (Fig. 17.2). In Washington state, where take-all suppressive soils are well characterized, there is compelling evidence that strains of *Pseudomonas* spp. that produce the antifungal metabolite 2,4-diacetylphloroglucinol, which is inhibitory to *G. graminis* var. *tritici*, are largely responsible for take-all decline.

Utilizing naturally occurring suppressive soils by planting a crop in an appropriate soil type is one way to take advantage of suppressive soils. Another idea is to manipulate the system and transfer the microbes that confer suppression. A simple alternative that is suitable in some cases is to add organic amendments to the soil. Although the benefits seem to be system specific, if green organic material is added

to some soils, the activity of resident antagonists is increased and pathogens can be suppressed, often by antibiosis. Such an effect of boosting the activity of resident antagonistic microbes has also been shown when adding chitin from shellfish production or compost to soil (Campbell, 1989).

17.5 Biological control of plant parasitic nematodes

While many nematode species are free-living, some of the most important plant parasitic nematodes are quite sedentary, remaining closely associated with plant roots. Many plant parasitic nematodes have developed defenses for protection in the complex soil environment, such as burrowing within plant tissues or producing their own protective structures on plant roots. Thus, plant parasitic nematodes are very often difficult to control, there being virtually no chemical treatments for control aside from soil fumigation. Availability of antagonists that breach nematode defenses would be extremely helpful.

Numerous specialist and generalist microorganisms are known to attack plant parasitic nematodes within the soil and some soils are generally suppressive to certain plant parasitic nematodes. However, relatively little is known about the ecology of these antagonists and the conditions necessary for them to provide effective control, largely due to difficulties in quantification and visualization of microbes around plant roots. Major groups of antagonists include (1) obligate pathogens such as the bacterium *Pasteuria penetrans* and the fungus *Hirsutella rhossiliensis*, (2) facultative pathogens such as fungi in the genus *Verticillium*, *Paecilomyces lilacinus*, and the nematode-trapping fungi (Fig. 16.2), (3) bacteria that inhabit the root zone, called rhizobacteria, that often produce toxins or modify root exudates used by nematodes, and (4) competing microorganisms that colonize roots, such as mycorrhizal fungi.

Let's consider the potential of these first two groups for commercial production. First, obligate pathogens do not grow outside of their host so large numbers would have to be released, but obligate pathogens are notoriously difficult to culture. Obligate pathogens also often have a narrow host range, making the activity of a commercial product very specific. While there are environmental advantages to specificity, less of the product would be sold compared with an antagonist with broader specificity. Facultative pathogens, on the other hand, could increase in the soil on their own and perhaps less material would have to be applied. Facultative pathogens are usually easier to mass-produce and are less specific.

The soil environment and host plant can have a strong influence on nematodes as well as on the community of antagonists in the rhizosphere. As an example, in soils where the cereal cyst nematode (*Heterodera avenae*) is surprisingly not abundant, nematode

populations appear to decline over a period of a few weeks after the new generation of females and eggs are exposed in the root zone (Kerry, 2000). It was hypothesized that during this time of exposure, females and eggs might be attacked by fungal pathogens. Modeling the dynamics of various fungi in the soil, the facultative pathogen *Verticillium chlamydosporium* was found to increase in density when nematodes had increased and vice versa. Nematode-suppressive soils have been identified numerous times but further study has not always attributed the antagonistic activity to the same natural enemy. These findings suggested that *V. chlamydosporium* could be reacting to nematodes in a density-dependent manner and thus regulating pest densities. However, density dependence in other fungi that are facultative parasites, such as the nematode-trapping fungi, has been difficult to demonstrate and densities of organisms potentially competing with nematodes are not always associated with nematode densities.

There are currently few commercial products for biological control of plant-parasitic nematodes and their use to date is limited. The field is presently advancing through the identification of key antagonists and study of their ecology. As examples, the facultative fungal pathogen *Paecilomyces lilacinus* is an egg parasite of root-knot as well as cyst nematodes and also attacks fungal root pathogens. The rhizosphere-dwelling bacterium *Burkholderia cepacia* is an aggressive colonizer of the root zone and outcompetes plant-pathogenic fungi as well as plant parasitic nematodes. As an alternative approach, researchers have discussed methods for improving soil to conserve and enhance antagonists of nematodes, but strategies are still being investigated.

FURTHER READING

- Becker, J. O. & Schwinn, F. J. Control of soil-borne pathogens with living bacteria and fungi: status and outlook. *Pesticide Science*, **37** (1993), 355–363.
- Boland, G. J. & Kuykendall, L. D. (ed.). *Plant-Microbe Interactions and Biological Control*. New York: Dekker, 1998.
- Butt, T. M., Jackson, C. & Magan, N. (eds.). *Fungi as BioControl Agents: Progress, Problems and Pitfalls*. Wallingford, UK: CABI Publishing, 2001.
- Campbell, R. *Biological Control of Microbial Plant Pathogens*. Cambridge: Cambridge University Press, 1989.
- Cook, R. J. Making greater use of introduced microorganisms for biological control of plant pathogens. *Annual Review of Phytopathology*, **31** (1993), 53–80.
- Harman, G. E. Myths and dogmas of biocontrol: changes in perceptions derived from research on *Trichoderma harzianum* T-22. *Plant Disease*, **84** (2000), 377–393.
- Hoitink, H. A. J. & Boehm, M. J. Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. *Annual Review of Phytopathology*, **37** (1999), 427–446.
- Kerry, B. R. Rhizosphere interactions and the exploitation of microbial agents for the biological control of plant-parasitic nematodes. *Annual Review of Phytopathology*, **38** (2000), 423–441.

- Mathre, D. E., Cook, R. J. & Callan, N. W. From discovery to use: traversing the world of commercializing biocontrol agents for plant disease control. *Plant Disease*, **83** (1999), 972–983.
- Stirling, G. R. *Biological Control of Plant Parasitic Nematodes: Progress, Problems and Prospects*. Wallingford, UK: CAB International, 1991.
- Tjamos, E. C., Papavizas, G. C. & Cook, R. J. (ed.). *Biological Control of Plant Diseases: Progress and Challenges for the Future*. New York: Plenum Press, 1992.
- Weller, D. M., Raaijmakers, J. M., McSpadden Gardener, B. B. & Thomashow, L. S. Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annual Review of Phytopathology*, **40** (2002), 309–348.
- Wojciech, J. J. & Korsten, L. Biological control of postharvest diseases of fruit. *Annual Review of Phytopathology*, **40** (2002), 411–441.

Part V

Biological control: concerns, changes, and challenges

Where is biological control used today? First, we will discuss safe use of biological control to prevent or minimize non-target effects and assure the public that biological control practitioners have learned from past mistakes and will not repeat them. Next, we will review the many and varied ways in which biological control is put to use today.

Safe biological control

... ladybirds supplied the first case of classical biological control and continue to be successfully used against pests. However, this can result in a conflict of interest between conservationists and biological control practitioners. There are theoretical grounds and some empirical evidence to indicate that introduced species of ladybirds might adversely affect the abundance of rare species of prey and native species of ladybirds. However, there is not good evidence of this having occurred and the most likely alternative to biological control, chemical control, is undoubtedly considerably more damaging to the environment.

(Dixon, 2000)

Issues regarding the safety of biological control introductions are aimed at identifying and preventing unintended, deleterious effects. Biological control programs strive to impact pests, or so-called “target” species, using natural enemies. However, due to previous practices in biological control when concerns and priorities differed, some natural enemies were released that affected non-target organisms, in particular endemic invertebrates. Bringing to light these effects has created controversy among many, including both conservationists and practitioners of biological control. In fact, in this era of excessive reliance on chemical pesticides, practitioners of biological control have long worked under the impression that they were helping the environment in developing methods for a safer type of pest control. Now, they must alter some methods and justify already-conscientious practices so that biological control is viewed once again as being an environmentally safe type of pest control, as well as being effective. In this chapter, we will examine some of the examples of non-target

effects of natural enemies, discuss why non-targets have been affected, and describe how non-target effects are now being prevented.

18.1 | Importance of non-target effects

Most natural enemies have hosts that they preferentially attack but also have species that are second options when the most commonly affected, preferred host is not available. To make sure about these second options, natural enemies that are considered for biological control of pests are first tested to ensure that they do not target humans as a first or second alternative or even as a distant runner-up. Very few programs are aimed at controlling vertebrates; therefore, natural enemies that would affect any vertebrates are rarely considered for biological control (see pp. 98–99). The acute and chronic toxicities toward vertebrates associated with some synthetic chemical pesticides are unheard of for biological control agents. However, a few of the microbes used for biological control of plant pathogens can act as opportunistic pathogens of humans, affecting immune-compromised humans (Handelsman, 2002). Due to the variability in specificity among microbial strains, researchers make sure that the microbial strains used for biological control are not pathogenic to humans. Perhaps a greater concern regarding effects of biological control agents on vertebrates are immune responses by workers regularly exposed to massive amounts during mass-production or regularly exposed during field work. Precautions have been developed to make sure workers are not hypersensitive and to prevent high levels of exposure, if an agent could provoke such a response (see National Research Council, 1996).

The vast majority of organisms of concern regarding unintended effects of biological control programs are invertebrates and plants. After ensuring that natural enemies for biological control would have no impact on vertebrates, the first group of non-target species to be considered are always beneficial organisms such as crop plants, honeybees (*Apis mellifera*), silkworms, parasitoids, and predators that humans work to maintain in the environment. Close watch has always been paid prior to release to prevent effects by natural enemies on beneficial non-targets. The potential for effects on beneficial organisms by a natural enemy considered for release are tested prior to release. In recent years, the spectrum of species being evaluated as non-targets has expanded. With growing interests in protecting the biodiversity of native ecosystems, effects of biological control agents on the native flora and fauna, and especially on threatened or endangered and rare species, are of concern.

Concerns about safety have focused on long-term establishment through classical biological control, largely because the results of these releases are considered irreversible. In particular, non-target effects to economic plants have always been scrutinized closely during programs for the biological control of weeds to make certain that

the natural enemies being investigated will not attack economically important plants, such as crops. Effects from inundative or inoculative releases are considered temporary, occurring during the period of time when natural enemies that are released are abundant, but then declining afterward (Box 18.1). However, there has also been some discussion regarding exotic natural enemies released inundatively that then might persist in the environment. Non-target effects of conservation biological control have not been evaluated because natural enemies are not released and, instead, habitats are manipulated. Because many microbes are considered ubiquitous (see Chapter 15), biological control programs utilizing microbes to control plant diseases have focused little on effects on the flora and fauna.

Box 18.1 | **Safety of *Bacillus thuringiensis*** (Glare & O'Callaghan, 2000)

The most widely used biopesticide, *Bacillus thuringiensis* or Bt, merits discussion regarding safety, a subject that has been investigated extensively. Since this pathogen is a common soil inhabitant worldwide, the issues are whether higher doses than naturally occur will have deleterious effects. Of course, results from any studies must be viewed with regard to whether the dose being used for studies reflects doses that could be encountered in nature. As everyone knows, while nutrients at low concentrations are needed by our bodies, high doses can be toxic. In addition, would the organisms of concern ever be exposed to the high doses of Bt that can be used in laboratory tests?

Of greatest concern is the effect of Bt on non-target invertebrates. Individual strains of Bt have very different specificities and there are multitudes of strains. Surprisingly, Bt almost exclusively affects phytophagous organisms and never carnivorous hosts, therefore having little direct impact on predators. Overall, Bt has rarely been found to harm directly any beneficial invertebrates. Bt can indirectly impact beneficials by robbing them of their prey or hosts but, for integrated control with the goal of lowering pest populations, Bt is useful in the majority of circumstances. As for honeybees and earthworms, the Bt strains not producing exotoxins that are used for pest control have shown no adverse effects.

Studies have addressed the diversity of species that might be impacted by use of Bt in non-crop areas hosting native species, such as aquatic habitats that would be sprayed for mosquito control or forests that could be sprayed to control outbreak populations of caterpillars. No study has ever detected non-target species at risk of local extinction after Bt applications. Extensive studies have been conducted evaluating the effects of Bt active against caterpillars on the diverse caterpillar fauna living in the same habitat as gypsy moths in the eastern USA. In West Virginia, Linda Butler and associates (1995) found that the populations of native caterpillars present when Bt sprays were applied declined in the year of application. Dave Wagner and associates (1996) took this one step further and followed populations of caterpillars in forests during a Bt treatment year as well as in the 2 years following a spray. During the treatment year, 19 of the 20 common caterpillar species on foliage decreased slightly but most species recovered the year after. In fact, for gypsy moth, it has been suggested that not controlling outbreaks in some way would have

at least as great an impact on the native fauna as spraying Bt, due to alterations in the habitat when high populations of gypsy moth larvae eat all of the leaves in the forest during spring.

Another part of risk evaluation is determining persistence. Bt is a spore-producing bacterium and spores do an excellent job of persisting in protected locations such as the soil. The Bt toxin alone survives only a relatively short time. Bt spores persisting in an area do not seem to pose a problem because this pathogen is found worldwide and does not cause epizootics on its own.

As for effects on mammals, any proven cases of Bt causing clinical disease in humans are very rare and extensive safety testing using small mammals has rarely indicated any toxicity.

In summary, studies have proven that Bt can be considered an environmentally friendly means for pest control. When the safety of Bt has been closely considered with a critical eye, it has been judged that any organism so widely disseminated with virtually no adverse incidents over several decades is likely to be relatively safe (Dixon, 1994).

For classical biological control, introducing a specialized exotic natural enemy to control an introduced pest is considered much safer than introducing an exotic natural enemy to control a native pest. In the latter case, the “new association,” the exotic natural enemy must have a somewhat broader host range so that it will accept the native pest as a host, although it has not previously encountered that pest species. The extension of this argument is that if the exotic natural enemy attacking the native will accept this pest with which it did not evolve, what other species might it accept as host or prey? However, detailed evaluations of non-target effects due to new versus old associations have not yet been undertaken to demonstrate whether this concern is well-founded.

18.1.1 Host specificity

Central to concerns about non-target effects is understanding the host specificity of a natural enemy, the range of hosts or prey that are used for growth and development. Of course, this term refers to the range of plant species attacked by an herbivore or plant pathogen as well as the range of host or prey animals attacked by predators, parasitoids, and pathogens. The breadth of hosts utilized by a natural enemy can be categorized along a continuum ranging from monophagous to oligophagous to polyphagous. Control programs generally strive to use natural enemies that are monophagous, those species utilizing only one host or prey species. However, efficient monophagous natural enemies that can be manipulated by biological control programs are not always available. Sometimes this is because they do not exist in the community of natural enemies associated with the pest. Also, not all species considered for biological control are easy

to culture successfully and sometimes a monophagous species that appears promising is just too difficult to work with.

In fact, during the earlier years of biological control, broad host specificity in natural enemies was not always considered an impediment to their use. Pests at outbreak densities can cause extensive damage and effects of natural enemies on non-target invertebrates were often not even considered during attempts to control devastating pests. When less was known about the environment and humans were struggling to battle pests to provide food and shelter for themselves, a broad host range could even be considered beneficial because then the natural enemy population would not become rare or extinct in that area after pests were under control.

18.2 | Reasons non-target effects have occurred

Relative to the total numbers of classical biological control releases, there are few examples of non-target effects due to biological control agents. An analysis of 5,279 classical biological control releases of insect natural enemies to control insect pests found that non-target effects or potential non-target effects were recorded from only 1.7% (Lynch & Thomas, 2000). In only 17 introductions (of 14 agents) out of the total 5,279, were non-target effects considered serious, with a mortality rate greater than 40% or with direct evidence of population decline. In the most extreme case, the tachinid parasitoid *Bessa remota* released against the coconut moth on Fiji in 1925 (see Box 3.2) attacked a related moth (*Heteropan dolens*) causing apparent extinction over a small area. A similar study of biological control of weeds evaluating 400 insects, mites, and fungi released for classical biological control reported non-target effects for only 0.5% (Fowler *et al.*, 2000). Other estimates of non-target effects are higher. Hawkins and Marino (1997) found that 16% of 313 parasitoids established in the eastern USA have been recorded parasitizing native species of hosts. Pemberton (2000) found that 12.8% (15/117) of agents established for biological control of weeds adopted native host plants.

Some critics say that these low percentages are due to lack of documentation. Although many classical biological control programs are evaluated directly after release to document establishment and efficacy of natural enemies that were released, later post-release monitoring has often been minimal, if occurring at all. In addition, after releases, the natural enemy and pest are generally monitored and only rarely are non-target species monitored. To make before and after comparisons more difficult, there is rarely much objective information about the abundance patterns for many species of the native flora and fauna. Thus, if native species increase or decrease in abundance after releasing a natural enemy, we often cannot say whether yearly changes in the densities of native species are normal or not.

Thus, there are few well-documented examples that prove whether non-target species were affected by biological control agents or not.

Among the examples where non-targets have been affected by natural enemies, there are several general scenarios to explain why natural enemies that would later cause non-target effects were released.

18.2.1 Changing priorities

Today, we are witnesses to results from biological control programs made in previous times. In some cases, decisions to introduce natural enemies were made when any concern regarding effects on the native flora and fauna were overshadowed by the problems caused by the pest. This certainly occurred in earlier years when vertebrate predators were released for pest control. Although vertebrate predators, with the exception of fish, are no longer released, these early introductions remain, providing examples of classical biological control introductions resulting in bad side-effects. One example of just such a disastrous release is the cane toad, *Bufo marinus*, introduced to numerous areas including Australia in the 1920s to 1930s (Lewis, 1989). These toads were introduced to control greyback (*Dermolepida albohirtum*) and the frenchi (*Lepidota frenchi*) cane beetles (Scarabaeidae) feeding on sugar cane at a time when worries about poor yields in sugar cane controlled decisions. Insufficient effort was made to understand the biology of the toad before release. It turned out that *B. marinus* is a very effective predator with an incredibly wide host range. The toads would eat the sugar cane scarabs if they were in the same locations at the same times, but they are not. When cane toads eat insects, they eat those on the ground but the sugar cane scarabs the toads were supposed to attack were high on the plants. When the adult scarabs are in the cane fields, there is no cover on the ground and cane toads, avoiding sunlight, can't be found in fields without cover. The cane toads therefore provided no control of the target pests. However, this toad readily became established and increased to large numbers, eating many endemic species, eventually radically changing the community level food webs as they spread in eastern Australia.

In the early 1900s, gypsy moth caterpillars were causing extensive defoliation year after year in New England. Controlling this forest pest was very difficult and numerous classical biological control introductions were made. One of the natural enemies that was introduced from Europe, a parasitic fly, *Compsilura concinnata*, that attacks larger gypsy moth larvae became established (Fig. 18.1). This is a species of tachinid with adult females that can pierce the host cuticle and inject living larvae into hosts; this is possible because eggs hatch within the mother. Researchers knew that in Europe, this fly was highly polyphagous. At that time, the craze to control gypsy moth was such that polyphagy was not considered a reason to prohibit release of this parasitoid. After establishment, this fly regularly attacked gypsy moth larvae but, although it can have an influence, *C. concinnata* was

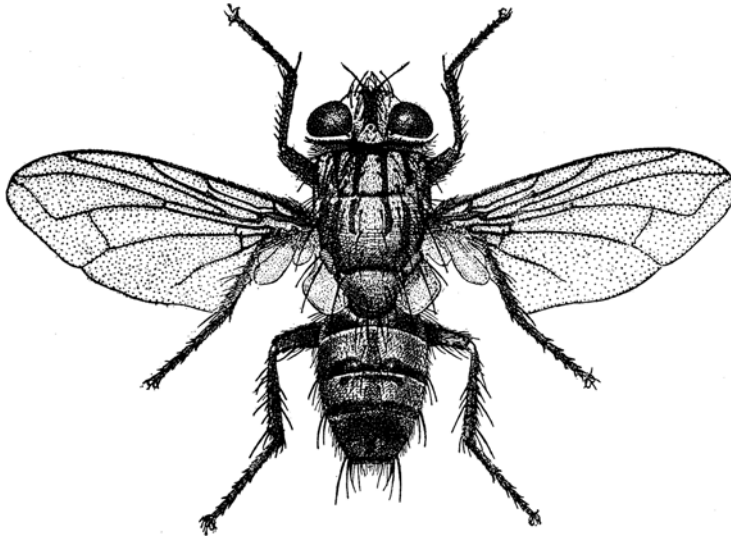


Fig. 18.1 Adult female of the tachinid parasitoid *Compsilura concinnata*, first introduced to North America for control of gypsy moth, *Lymantria dispar*, in 1906. Approximately 7.5 mm long. (Culver, 1919.)

never considered as regulating gypsy moth population densities. In recent years it was noted that the populations of wild silk moths (Saturniidae) in New England were not as abundant as recorded in literature at the turn of the century. Jeff Boettner and Joe Elkinton (Boettner *et al.*, 2000) hypothesized that *C. concinnata* could have influenced silk moth densities. They investigated this by placing caterpillars of the native North American cecropia moth (*Hyalophora cecropia*) in the field and then watching to see if they were parasitized. The cecropia caterpillars were parasitized by *C. concinnata* at high enough levels to strongly suggest that the activity of this parasitoid could be responsible for the present-day low densities of cecropia moth populations. This is clearly an example of changing priorities over time; maintaining the biodiversity of the endemic moth fauna of New England is a priority for numerous people now but this certainly was not a priority that carried weight in the early 1900s although larvae of wild silk moths were commonly collected by hikers and others who reared the moths as a hobby. In fact, *C. concinnata* has been released from 1906 to 1986 to control 13 different pest species but recently the tide has turned. It is doubtful whether *C. concinnata* would be released today since it is such a generalist.

Another example of changing priorities is the release of the flower-head weevil, *Rhinocyllus conicus*, for control of introduced thistles (Box 18.2). It is generally thought that the more specific a natural enemy for use in classical biological control, the better the resulting control and the less chance of non-target effects. However, this weevil example demonstrates that to prevent non-target effects it is also important to consider which species are present in the area of release. Although this weevil does not have broad tastes, it feeds on thistles in the genus *Cirsium*. North America has 90 species of *Cirsium* that are widespread and several of these species occur in the area where this

Box 18.2 | Wily weevils that like thistle seeds

Exotic thistles that were accidentally introduced to North America were good competitors and became problems in agriculture, especially in rangelands where they outcompeted the native grasses eaten by cattle and horses. A Eurasian weevil, *Rhinocyllus conicus*, whose larvae develop while feeding on seeds within developing thistle seed heads, was studied as a potential classical biological control agent. Pre-release testing conducted in Europe demonstrated that this species was oligophagous and, although it appeared to prefer European thistles, it would also oviposit and develop on other closely related thistles. Native thistles in North America occurred within the same thistle genera as those successfully attacked by this weevil in Europe (*Cirsium* spp.). However, largely based on concerns over the extensive damage caused by the introduced thistles, the decision was made to release *R. conicus* despite potential non-target effects. *R. conicus* was introduced as a classical biological control agent in Canada in 1968 and the USA in 1969. This weevil was considered quite an effective natural enemy for controlling the introduced thistles.



The flowerhead weevil, *Rhinocyllus conicus*, (c. 10–15 mm in length), native to Eurasia has been introduced to Argentina, Australia, New Zealand, and North America for control of weedy thistles. (Illustration by Alison E. Burke.)

However, in 1987 it was found that *R. conicus* was developing within seed heads of 17 California species of *Cirsium*, including rare species, along with the European thistles. In 1997, the population level implications were evident when it was reported that among the North American native thistles, an 86% reduction in viable seed was found in Platte thistle (*Cirsium canescens*), and a 72% reduction in wavyleaf thistle (*C. undulatum*) (Louda et al., 1997). This example repeats a scenario where decisions made for agronomic purposes at a time when environmental side effects were not considered as important have later been challenged when the natural enemy that was released was criticized because it affects North American natives.

weevil was released. Impact of *R. conicus* on native North American thistles has resulted in controversy over the choices that were made when releasing this weevil.

18.2.2 Insufficient information about a natural enemy

The cane toad provides an excellent example of lack of knowledge regarding the ability of a natural enemy to control a pest. This example occurred long ago and can perhaps be explained by difficulties in communication and in conducting scientific studies during those times. Unfortunately, non-target effects from releases as late as 1977 also occurred because insufficient information was distributed about a natural enemy. The giant African snail *Achatina fulica* was introduced to Tahiti as a source of food. It is voracious, multiplied quickly, and became an agricultural pest (Murray, 1993). One resident described taking two wheelbarrow loads of snails from the wall of his house in one day. The predatory land snail *Euglandina rosea* is native to Florida and Central America and was introduced to the Pacific island of Moorea, 12 miles from Tahiti in the Society Islands, to control the giant African snail. This predator had been introduced to Hawaii to control this same pest in 1955 but had not been effective and had been implicated as one of several causes of the decline and extinction of the native tree snails that it ate instead of the giant African snails. This experience did not prevent the introduction to Moorea, perhaps because the Mooreans were not aware of the overall effects of this predator in Hawaii. In 1977, *E. rosea* was introduced to Moorea and it took 10 years to spread across the island. There is no proof that this predator controlled the African snails but it certainly liked to eat the native Moorean snails. The entire genus *Partula* of native Moorean snails could no longer be found by 1987. Certainly, this is a classical biological control introduction that was not associated with enough testing to determine whether the natural enemy was effective at controlling the pest, let alone whether it would affect native species. Such a dramatic impact on the unique native snail fauna of Moorea has not been excused by the environmentally aware public.

Classical biological control programs are often mounted to control newly introduced species that are considered important pests. In some programs, many species of natural enemies have been

introduced over a relatively short time period in response to strong pressures from fears about damage from a new invader. One example is the Russian wheat aphid (*Diuraphis noxia*) that was introduced to the USA. A classical biological control campaign was mounted and between 1986 and 1993, 29 new species of insect predators and parasitoids were introduced for control. This program is not unique in its intensity. Instituting requirements to answer today's non-target concerns before such an abundance of species could be released would slow such programs and perhaps lead to fewer natural enemies being introduced or at least they could not be released quickly because more extensive host-specificity testing would require more time (see below).

18.2.3 Fragile ecosystems

Non-target effects certainly would have greater impacts in fragile ecosystems, with less-complex food webs. In particular, islands often host fewer species of endemic plants and animals than mainlands and island endemics are thought often to have fewer preformed defenses against natural enemies. Some of the most strident voices criticizing classical biological control use examples from Hawaii. This is an extreme example because not only is Hawaii an island but, due to its location as a stopping place for shipping, especially when the world relied on sailing ships, many pests have been introduced and many natural enemies have been released to control these introduced pests.

More classical biological introductions have been made in Hawaii than anywhere else in the world. Between 1890 and 1985, 679 species of organisms were introduced to Hawaii for biological control of insects, weeds, and other species and 243 became established (Funasaki *et al.*, 1988). Since 1900, the majority of natural enemies released (71.6%) have been parasitoids and predators released to control insects; these figures are not unusual, being similar to percentages of classical biological control releases worldwide. Relative to this number of releases, the number of negative effects is not far from the worldwide averages. Few of the reported occurrences of non-target effects are based on objective data. In fact, in most of the cases, it is impossible to tell whether the decline of a non-target was caused by an exotic natural enemy purposefully released, by competition from an invasive species, or by some other factor limiting the populations such as habitat depletion or degradation.

The beautiful green koa bug (Fig. 18.2) that feeds on the native koa trees of Hawaii provides an example of the difficulties in documenting non-target effects. An agricultural pest, the southern green stink bug (*Nezara viridula*), was accidentally introduced to Hawaii in 1961 and, by 1963, two species of exotic parasitoids were being introduced for control. One was a parasitic fly (Tachinidae) attacking late-stage nymphs and adults (*Trichopoda pilipes*) and one was a tiny egg parasitoid (*Trissolcus basalis*). The southern green stink bug and the koa bug belong to closely related families of true bugs, the

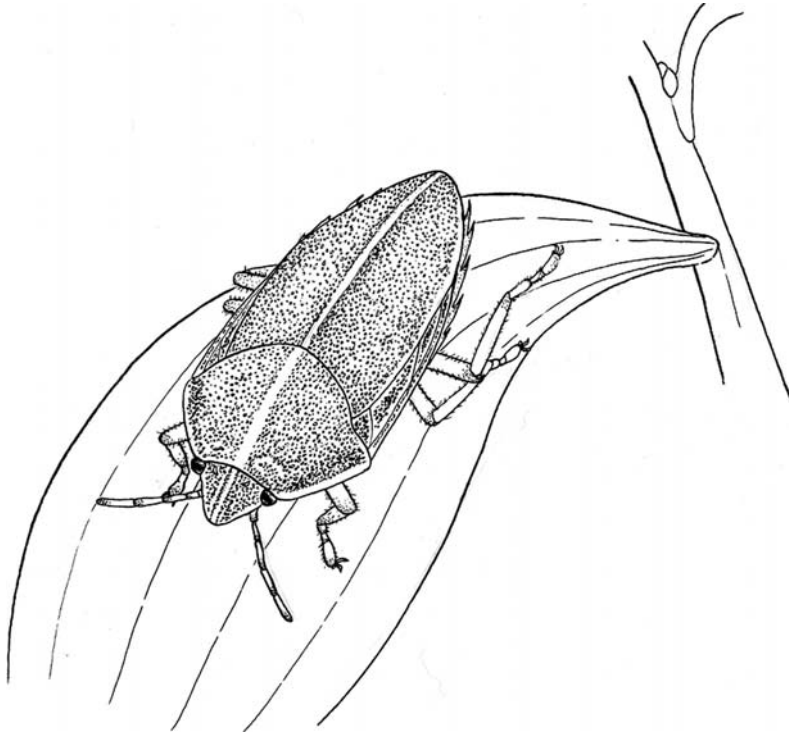


Fig. 18.2 The native Hawaiian koa bug, *Coleotichus blackburniae* (c. 15 mm in length). (Illustration by Alison E. Burke.)

Pentatomidae and Scutelleridae, respectively. In 1991, largely based on records from museums, it was stated that the parasitoids introduced to control southern green stink bug had caused the decline of the koa bug. However, an in-depth study by Peter Follett started out by finding that koa bugs could be quite abundant if you went to the field looking for them in the correct places (Follett *et al.*, 2000). Life table analyses demonstrated that generalist predators, not introduced for biological control, cause the highest mortality to koa bugs in all habitats on Hawaii (Louda *et al.*, 2003). However, the introduced egg parasitoid was found having a significant impact on koa bug populations in one of the many native habitats sampled. In this case, evaluating non-target effects of biological control introductions is complicated because the releases were conducted 30 years ago. There is no clear answer regarding whether koa bug populations have declined because no one quantified typical koa bug population densities before releases. If koa bug populations did decline at some time after the natural enemies were released, the cause of the decline was not documented. Most of the evidence for a decline in koa bug populations comes from Oahu, the most heavily populated island in Hawaii, and low densities of koa bugs on Oahu could easily be due to lack of native habitat and invasions by generalist predators. Since the biological control agents that were introduced in 1963 attacked koa bugs in the laboratory before introductions, this study shows that pre-release testing predicted that non-target impacts would occur.

18.2.4 Dispersing natural enemies

A few examples have shown that natural enemies can disperse unexpectedly. A caterpillar from Argentina, *Cactoblastis cactorum*, was introduced in Australia in 1926 to control species of the cactus *Opuntia* that had been introduced and had increased to create impenetrable, thorny thickets over huge areas (see Box 13.1). Amazing levels of control were recorded in Australia, where *C. cactorum* virtually cleared enormous areas of this weed. Based on this success, in 1957 and 1960 this caterpillar was introduced to several islands in the Caribbean. This cactus family has no native species in Australia and *C. cactorum* is quite host specific so there were no non-target concerns in Australia. However, this was a different picture in the Caribbean because there are many species of *Opuntia* that are native to the continental North America. In these Caribbean islands, the target weeds were primarily weedy native *Opuntia* but there are also non-weedy *Opuntia* species in the Caribbean Islands as well as in Mexico and North America. No one knows how it traveled, but in 1989, caterpillars of this weed control agent were found in Florida. Closer observation documented *C. cactorum* attacking all six species of *Opuntia* native to Florida and, of special concern, this includes the rare Floridian semaphore cactus (*Opuntia corallicola*). The semaphore cactus is rare due to habitat loss because it lives along the increasingly developed Floridian coast. However, the fact that *C. cactorum* will attack this rare cactus certainly is problematic because the few plants remaining must now be protected. Of more serious concern is the fact that this genus of cactus is diverse in the southwestern USA and Mexico and, in Mexico, the cactus pads (nopales) and fruit are used as food. If or when *C. cactorum* disperses to the southwestern USA and Mexico, *C. cactorum* may need to be controlled both to maintain biodiversity and to protect plants of this genus used as a source of food.

18.3 Direct versus indirect effects

Non-target impacts can potentially take two different forms. (1) Natural enemies can impact non-target as well as target species by using these as hosts or prey; this would cause a direct impact. (2) If a natural enemy perturbs something in an ecosystem, which in turn impacts a third species, an indirect effect has occurred. Indirect effects are especially troublesome because they are as difficult to prove as they are to predict. One example of an indirect non-target effect would be competition between exotic and native natural enemies. Generalist lady beetles that have been introduced for aphid control can be so effective that they outcompete native lady beetles. One of these biological control agents, the seven-spotted lady beetle (*Coccinella septempunctata*), eats lady beetles as well as aphids. It is thought that indirect effects of introductions of exotic lady beetles caused decreases in population densities of native North American lady beetles, such

as the nine-spotted lady beetle (*Coccinella novemnotata*), after these introduced generalists became established.

18.4 | Predicting non-target effects

For any natural enemy that is being considered for release, the host range must be considered along with efficacy in controlling the pest. Predicting non-target effects is not especially simple and practitioners of biological control have learned that several types of evaluations are required. Researchers studying biological control of weeds have far more extensive experience with host-range testing than other practitioners of biological control. From early times, programs for biological control of weeds have always included non-target testing to assure that natural enemies released would not eat crop plants. Host-range testing can require several tiers of studies and requirements will differ for different types of biological control (Box 18.3). For example, natural enemies that are insects require testing that takes behavior into account, offering choices of different life stages of natural enemies, such as adults versus immatures versus eggs. Natural enemies that are microbes would not require choice tests although different life stages of hosts could be tested at different doses because virulence could differ.

Box 18.3 | Testing host specificity

Some natural enemies feed on only one or a few hosts while others feed on numerous species. This variability in the number of species affected is seen across the diversity of natural enemies feeding on microbes, plants, or other animals. To determine whether non-target effects could occur, the host range of the natural enemy must be determined. However, it is often not so simple to determine host specificity with accuracy, especially for more complex organisms. Therefore, specific methods for testing have been developed in some cases.

Phytophagous natural enemies

Methods based on natural enemy biology have been developed for testing the host specificity of herbivorous arthropods. Different stages of insects can react differently to plant species and they do not always react in the same way. Both acceptance of plants for oviposition by adults and suitability for development of immatures must be tested. For example, adults of some species are known to lay eggs on plants that are not optimal for development of immatures. Conversely, plant species on which immatures can develop are not always accepted by adults for oviposition. How do you choose which plant species to test? It is common that many plant species are tested and, in particular, closely related plant species that occur in the potential release area must be tested.

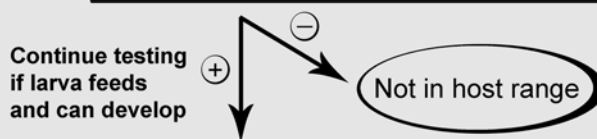
One strategy for determining the host specificity of natural enemies eating plants is called the “reverse-order” method; this method includes separate evaluations of oviposition by adults and development of immatures (Vapshere, 1989).

Step 1: Choosing test plants**Related to the weed****Not related to the weed**

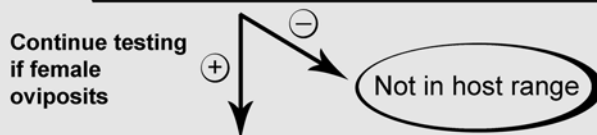
Plants not previously exposed to the natural enemy
 Plants with characteristics like the weed
 Plants attacked by relatives of the natural enemy
 Little known and rare plants

Step 2: Host range testing

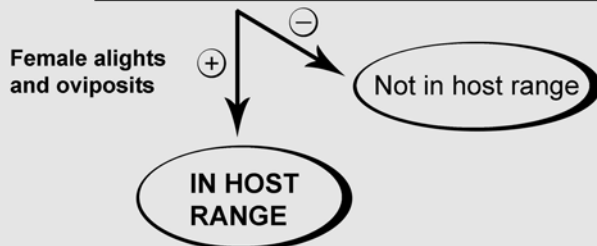
Test I: Larval feeding and development (no choice)



Test II: Adult oviposition (no choice)



Test III: Adult female (less confined choice test)



Testing sequence for evaluating the host range of herbivorous arthropods. Referred to as “reverse-order” because, in contrast to previous methods, ability of larvae to develop when fed on a plant species is tested before adult acceptance of the plant. (After Wapshere, 1989.)

First, larvae of the candidate agent are fed plants of interest, including the weed species as a positive control. The individual insects being tested are each fed species of plants separately, with no choice. If they will not eat or cannot develop successfully, then this phytophagous species could be used. However, plant species on which the insects can develop successfully from egg to reproductive adult next undergo a second tier of testing. The factors that adults use for deciding whether to oviposit on a plant or not are not especially the same as the factors necessary for successful larval development. Therefore, plants are next tested to see whether

adults will oviposit on them, without a choice. Results will indicate whether adults will ever accept the plant in question, an important fact if this plant species is scarce or any injury to it is not acceptable, as would be the case for rare and endangered plants. For the majority of plant species, the major concern is whether both oviposition and development would occur regularly. Only plants on which adults will lay eggs remain in the study for the third tier of testing when oviposition by adults is tested against a choice of numerous plant species. This entire test therefore identifies both whether immatures can develop and whether adults will lay eggs on plant species of concern.

Once scientists feel certain that they understand the host specificity of a natural enemy and that it seems to have a good chance of efficacy, a decision must be made regarding whether that agent should be released. At times such decisions are simple but certainly not always. Here, we should mention an example where a decision was not straightforward. A plant named *Echium plantagineum* was introduced in the 1800s to Australia where it grew too abundantly and was designated as a noxious weed in 1909 (Delfosse & Cullen, 1981). This plant is especially problematic because it has been linked with deaths of horses and sheep that have eaten it extensively; the pathology is due to liver damage from the pyrrolizidine alkaloids in this plant. Controlling this weed is difficult and classical biological control began to be investigated in 1972. A leaf-mining caterpillar (*Dialectica scalariella*) was introduced in 1980 and a stem-boring beetle (*Phytoecia coerulescens*) and two leaf beetles with root-mining larvae (*Longitarsus aeneus* and *L. echii*) were identified as promising control agents. However, biological control of this plant became a source of controversy. Although the common name used by ranchers for this plant was Paterson's curse, the common name used by beekeepers was Salvation Jane. The abundant flowers of this plant were considered a major source of nectar and pollen for honeybees. Whether to continue introductions of classical biological control agents or not was eventually decided by a panel including scientists, farmers, and beekeepers, as well as the general public. In the end, two inquiries found that control of the weed was in the national interest and the natural enemies were released.

Predators and parasitoids

For testing host specificity of predators and parasitoids, once again evaluations start under highly controlled situations. Understanding the behavior of these natural enemies that kill their individual hosts or prey requires attention to detail. We know that factors like the age and egg load of the natural enemy, densities of natural enemy and host or prey, length of exposure, level of host deprivation prior to testing, and type of testing arena can all affect whether a natural enemy will attack or not (Follett *et al.*, 2000). Taking into account such factors, once a micro-environment for testing has been developed and natural enemies and hosts/prey are available, the first type of study usually undertaken exposes appropriate stages of non-targets to natural enemies without offering a choice. Concurrently, to document that the natural enemy was healthy, a subset of individuals should be provided with hosts/prey that they are known to attack successfully. The next stage is to expose non-targets to natural enemies in a small arena, giving them a choice of the non-target versus the target. The last stage requires more effort but can potentially be more valuable, with studies conducted in a larger and more realistic arena, providing numerous non-targets and targets to try to simulate conditions that would occur in nature.

The choice of species to evaluate is not always straightforward. Creating a list of species for testing is difficult because it must be short enough to be practical but long enough to answer questions being asked about both economically and environmentally important non-targets. For many types of natural enemies, first those species closely related to the target pest should be tested, with successive species for testing being increasingly distantly related. This method, based on choosing species for testing according to their relation to the target, works well for natural enemies that only attack closely related species. Some herbivores feed on plants based on plant chemistry and for phytophagous natural enemies using chemistry for host discrimination, plants to test should instead be chosen based on the chemicals they produce. Ectoparasitoids are often associated with specific habitats so a list of species to test would include not only close relatives of the pest but also other species living in similar habitats in the release area, for example, invertebrates boring within wood, which would include taxonomically diverse organisms.

It is clear that what we actually want to know before deciding whether a natural enemy should be released is what the host range in the new area will be. How can we predict this before introducing a natural enemy to a new area? One clever approach has been to investigate the host range in the area of endemism, including thinking about those species that might be present but are not attacked. In fact, this has been done in biological control of weeds for many years. After the natural enemy has been accurately identified to the species level (which is not always so easy; see Chapter 3), a review of the literature will hopefully yield an additional list of known hosts in the area of endemism. However, such lists are frequently based on random field collections and are often incomplete. Information about the host range in the area of endemism is critical for situations where the natural enemy does not already occur in the area for release, for example with classical biological control introductions and releases of genetically engineered organisms, when investigation of the area of endemism is the only recourse. Such surveys can provide very important information about specificity in the field. Although the community in the area of endemism could differ substantially from that in the area of release, an evaluation in the field in the area of endemism would give a first glimpse of how narrow or broad the host range of the natural enemy is in nature.

Next, controlled tests under optimal conditions are conducted in quarantines, laboratories, or caged areas, depending on the types of tests and natural enemies. Sometimes, testing is also done in the native area, where quarantine is not needed. Data from controlled exposures will yield the physiological host range, or those hosts that could potentially be used by the natural enemy for development. For many species of natural enemies, all of the prey or hosts that might be attacked under controlled experiments in the laboratory will not be attacked in the field. The gypsy moth fungal pathogen *Entomophaga maimaiga* could infect 28 out of 78 species of non-target caterpillars

in the laboratory but only three of these were found infected in the field, and infection was at very low levels (Hajek *et al.*, 2003). This is in part because the gypsy moth has unusual behavior for a caterpillar. Larger larvae rest in the leaf litter and, in doing so, become exposed to the abundant spores of this fungal pathogen in the surface layers of the soil. Few of the caterpillar species that are potentially susceptible to this fungus ever venture to the ground for long periods of time, let alone spend all day long there as gypsy moth caterpillars do. The behavior and seasonality of the non-target can play a large part in whether that species is attacked by the natural enemy in the field. The ecological host range, or those species actually attacked in the field, can therefore be quite different from the physiological host range, but it is the ecological host range that is important for biological control purposes.

Of course, we are trying to predict the species that a natural enemy will attack in a new area. Information on the flora and fauna of the area for release, as well as contiguous areas where the natural enemy might disperse, is important for consideration. Concerns about non-target effects of agents for the biological control of weeds have been alleviated when herbivores specializing on a plant family that is not native to the release area are considered for release against an invasive weed belonging to that plant family. We have to make the best estimates based on appropriate information before making decisions about the potential non-target effects of natural enemies. Adding information about host range from the laboratory and from the area of endemism to knowledge of the ecology and behavior of the natural enemy and the species that might be affected in the area of release will help to arrive at the best prediction regarding non-target impact. Once this information is in hand, practitioners of biological control can make informed decisions about whether specific biological control agents should be considered for release. After release, field evaluations to determine non-target effects are critical so that we can match predictions with results and gain more information toward preventing non-target effects of biological control. Because natural enemy population densities can vary significantly from year to year, evaluations encompassing several years are preferable.

Indirect effects are much more difficult to predict and involve ecological investigations at the community level. Information about the community in which releases will be made may help to identify potential indirect effects. For example, introduced natural enemies could compete with native natural enemies or they could attack native natural enemies. Alternatively, introduced natural enemies could hybridize with natives, leading to changes in the genotypes present.

18.4.1 Will host specificity change?

Concern has been voiced regarding whether the host range of natural enemies will change after release. Biological control of weeds has undergone quite a bit of scrutiny regarding host ranges of natural enemies for potential introduction, but an in-depth analysis of

different programs found no evidence for increases in host ranges after introductions. Any changes that were reported were due to new associations (e.g., exotics were introduced that had not encountered certain host plants previously), errors in sampling, or changes in the ecology, such as increasing host densities. There have been a few instances when a natural enemy population was very abundant and the regularly used hosts (pests) had become rare due to overuse by the natural enemy and this resulted in utilization of a previously non-attacked host. This situation has been found to be transient, occurring only with abnormally high natural enemy densities, usually not long after natural enemies had become established. The natural enemies increased to large numbers due to an overabundance of hosts but then ran out of hosts through their own over-efficiency. This situation occurred when the lace bug *Teleonemia scrupulosa* was released against the weed *Lantana camara* in Uganda. When populations became very high, the bug began eating sesame, but this had not been predicted. However, feeding on sesame by this bug was transient. *T. scrupulosa* has now been introduced to a total of 27 countries and islands and has not been reported as a pest of sesame in any other instance.

Projects for the biological control of weeds in the USA, the Caribbean, and Hawaii, including 112 insects, 3 fungi, 1 mite, and 1 nematode, were evaluated by Robert Pemberton (2000). He found that all attacks on non-targets could have been predicted based on test results before organisms were released. Non-target effects were not due to changes in the host ranges of exotic natural enemies but instead, non-target plants that were attacked were all closely related to the target weed and occurred in the same habitat. Therefore, non-target effects could have been prevented by targeting weed species having few to no closely related species native to the release area. Thus, no studies have documented unpredictable changes in host range by natural enemies after their introduction. The key here seems to be that excellent knowledge of the host range of the natural enemy is required as well as the breadth of species occurring in the release area.

While a true change in host preference and acceptability for a natural enemy would require genetic changes, which are less likely, a change in the intensity of an association already in place is more possible. Generally, this could be a decrease in effectiveness of a natural enemy against a pest or an increase in resistance in that pest against the natural enemy and could therefore be called coevolution. The only example of this from biological control has been the coevolution documented between the myxomatosis virus and its rabbit host in Australia (Chapter 11). After release, the virulence of the myxomatosis virus decreased while rabbit resistance to the virus increased. This change is believed to have been partially driven by natural selection to enhance virus persistence. The mosquito-transmitted virus is only transferred from living infected rabbits to healthy rabbits so, if rabbits die quickly after infection, the virus has less chance of being transmitted. When the virus became less virulent, rabbits survived

longer after infection so there was a longer window for the pathogen to be transferred to a new host.

Therefore, as previously stated, there are no examples of changes by natural enemies to use new hosts that could not have been predicted based on the known host range of a natural enemy, either through investigating activity in the area of origin of the natural enemy or through pre-release studies of host specificity.

18.5 Preventing non-target effects

Non-target effects of natural enemies have been documented in the literature but since these have been few and far between, they were not a focus of attention until recent years. A debate about adverse non-target effects of classical biological control releases was really catalyzed only beginning in 1983. In 1983 and then 1991 the effects of exotic natural enemies on the native biota in Hawaii were summarized by Howarth. Then, the effects of the weevil *Rhinocyllus conicus* on thistles on the North American Great Plains were published in 1997 (Louda *et al.*, 1997), resulting in further attention to this issue.

Although the releases made in previous years remain, the scientific community has been addressing the concerns raised. Practitioners of biological control responded by spending more time evaluating potential releases and thus the rate of new natural enemy introductions has slowed. For example, in Hawaii, between 1900 and 1980, 3.8 species were introduced per year. However, this release rate slowed to 2.3 per year during 1980–1989 and since 1990 fewer than two introductions were made per year (Fig. 18.3). In the meantime, guidelines for determining the safety of natural enemies have been developed.

Different countries each have their own methods for determining the risks of releasing exotic natural enemies and how this is regulated. However, biological control using exotic species really requires international collaboration and releases can have international

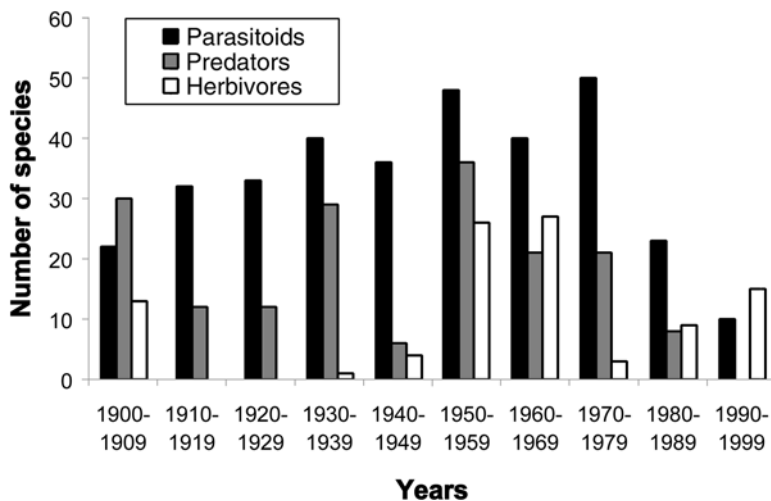


Fig. 18.3 Changes in use of classical biological control in Hawaii. (Updated from Follett *et al.*, 2000.)

repercussions. For countries on the same continent, a release could result in establishment across several countries. Therefore, in 1997 the Food and Agriculture Organization of the United Nations developed a Code of Conduct for the Import and Release of Biocontrol Agents to provide guidelines for releases of exotics.

The code was developed to provide a thorough framework for making decisions to promote safe use of exotic natural enemies for biological control. The code discusses exotic natural enemies being imported for potential release, and whether they would be used for any type of release of exotics: classical biological control, or inundative or inoculative releases. The code identifies the three main groups involved in a biological control release, the national authorities, the importers, and the exporters, and lists their respective responsibilities during different phases of an introduction program. One major responsibility is that the biology of the natural enemy and its potential risks must be summarized.

We have discussed methods for evaluating host specificity but how are results translated into decisions? Polyphagous agents or those requiring non-targets as part of their life cycle are rarely considered for release today. In this category, an important native parasitic wasp attacking gypsy moth larvae in Japan, *Glyptapanteles liparidis*, has several generations per year and overwinters in larval hosts. Gypsy moth has only one generation per year and overwinters as eggs. Therefore, after its first generation each year *G. liparidis* parasitizes other caterpillars. This species is, therefore, not being considered for importation to the USA and release; although it is an important natural enemy controlling gypsy moth in Japan it would certainly affect non-target caterpillars. Agents known to attack close relatives of native species that naturally occur in the release areas would also be a poor choice for introductions (see above). Attention should also be paid to whether the natural enemy could affect other species in the community in some indirect way. A parasitic wasp (*Diachasmimorpha tryoni*) was introduced to Hawaii early in the century for control of the Mediterranean fruit fly, or medfly (*Ceratitis capitata*). Later, a gall-forming fly (*Eutreta xanthochaeta*) in the same family as medfly (Tephritidae) was introduced for biological control of the weed *L. camara* in Hawaii. Unfortunately, this parasitic wasp now attacks the agent for biological control of the weed (Follett *et al.*, 2000). Once again, practitioners are learning from past mistakes and we've learned that a broader vision of possible ecosystem effects must be adopted when planning biological control programs.

18.5.1 Risk assessment

One question critical to evaluating risk is how much of an effect by a natural enemy is considered "adverse." This is a very subjective judgment. For ardent conservationists, an unacceptable risk could be one non-target individual being affected. However, what is certainly of more concern is whether the natural enemy becomes a source of significant and persistent mortality in populations of non-targets.

Clearly, what is needed is a method for making decisions about risk more objective. Overall risk can be considered the interaction between hazard and exposure. The physiological host range can be used to derive the hazard (those species that could be affected) and information about the ecology, biology, and behavior can be used to derive exposure information. Once risk has been identified, its likelihood and magnitude must be evaluated, followed by discussions of whether risks can be avoided or reduced.

Finally, the risk of using natural enemies must be weighed against the alternatives. No discussion of non-target effects is complete without mentioning that non-target effects caused by an exotic natural enemy cannot be considered alone. If decisions are made to control a pest and biological control is not chosen, it is likely that a different form of control, often use of synthetic chemical pesticides, will be used instead. In such a case, the range of flora and/or fauna affected will often be much greater than after release of an exotic natural enemy. Of course, another alternative would be to not control the pest at all. In such a case, an outbreak of the pest could have a strong impact on the community, affecting non-targets but in different ways. For example, an invasive weed not being controlled could out-compete the native vegetation and few endemic plants would remain. Along the same lines, if a pestiferous herbivore was eating all of the foliage on endemic plants, normal inhabitants of that ecosystem might not be able to survive under the altered conditions, either through loss of host plants or through physical changes to the microhabitat they require. Therefore, in deciding about using exotic natural enemies for pest control, the effects of the different alternatives on populations of non-targets should be considered. However, as an example of trust in the safety of biological control, host-specific biological control agents are usually considered first for pest control in areas where rare and endangered species occur.

FURTHER READING

- Follett, P. A. & Duan, J. J. (eds). *Nontarget Effects of Biological Control*. Dordrecht, NL: Kluwer Academic Publications, 2000.
- Hokkanen, H. & Hajek, A. E. (eds). *Environmental Impacts of Microbial Insecticides*. Dordrecht, NL: Kluwer Academic Publications, 2003.
- Lockwood, J. A., Howarth, F. G. & Purcell, M. F. (eds). *Balancing Nature: Assessing the Impact of Importing Non-native Biological Control Agents (An International Perspective)*. Lanham, MD: Entomological Society of America, 2001.
- Louda, S. M., Pemberton, R. W., Johnson, M. T. & Follett, P. A. Non-target effects – the Achilles' heel of biological control? *Annual Review of Entomology*, **48** (2003), 365–396.
- Thomas, M. B. & Willis, A. J. (1998). Biocontrol – risky but necessary? *Trends in Ecology and Evolution*, **13**, 325–329.
- Wajnberg, E., Scott, J. K. & Quimby, P. C. (eds). *Evaluating Indirect Ecological Effects of Biological Control*. Wallingford, UK: CABI Publications, 2001.

Present uses of biological control

19.1 | Using natural enemies alone?

Sometimes, biological control is a “stand alone” method and does not have to be used in conjunction with other methods. This is especially true for effective natural enemies being used against pests in uncultivated areas, aquatic weeds, rangeland weeds, or arthropod pests of ornamental plants or in forests, all of these being ecologically stable habitats usually requiring lower levels of management. For example, a classical biological control introduction to combat a forest pest, if effective, would not need further attention unless the balance established was disturbed. If use of natural enemies is intended as a “stand alone” strategy, generally the focus is on only one pest, as would be typical of a classical biological control introduction. However, use of natural enemies as a “stand alone” strategy is only possible if the target pest is maintained below the economic injury level without other interventions.

In pest control, it is common that numerous controls are used against one pest or pests are part of a pest complex requiring numerous types of control. Then, the different types of control that are used must be melded so that they are used in harmony and not at cross purposes. When pesticides must be applied to combat one pest, they should be applied so that they do not kill natural enemies controlling other pests in the same system. For example, while spraying fungicides on potatoes might control the fungal pathogen causing late blight (*Phytophthora infestans*), this also has the potential to kill the entomophthoralean pathogens that are controlling green peach aphid (*Myzus persicae*) populations on potato. To minimize negative impacts on natural enemies, selective pesticides can be sprayed in specific locations or at specific times to minimize effects on other natural enemies. A management strategy has been developed to address the complexities that arise in trying to use such an integrative approach and this has been called integrated pest management (IPM). IPM has been defined numerous times and one of these definitions with an ecological emphasis is provided below.

Integrated pest management is an ecologically based pest control strategy that relies heavily on natural mortality factors such as natural enemies and weather and seeks out control tactics that disrupt effects of these factors as little as possible. IPM uses pesticides, but only after systematic monitoring of pest populations and natural control factors indicates a need. Ideally, an integrated pest management program considers all available pest control actions, including no action, and evaluates the potential interactions among various control tactics, cultural practices, weather, other pests, and the crop to be protected.
(Adapted from Flint & van den Bosch, 1981.)

Although the roots of IPM were agricultural, uses today have spread through control of many different pests. In New York State, particular attention is now being paid to urban IPM programs, because many urban areas receive high doses of pesticides with high potential for human exposure.

19.2 | Control through managing pests

While this definition for IPM is somewhat long and complicated, the basic goal is to use control tactics against pests only when necessary. This approach is thus called management because sometimes the decision is to employ no control tactics at all and then the term “control” is somewhat inappropriate. IPM suggests using methods for control only if the pest population is causing damage above the economic injury level (see Chapter 2). IPM thus always requires a good understanding of the pest system. To use an IPM approach, methods for determining pest densities and knowledge of pest densities that cause economic damage are considered optimal. IPM programs are composed of six basic elements, with biological control fitting in as one of the different control tactics that can be used if control is necessary (Table 19.1).

A critical part of IPM is monitoring the pest population; this is referred to as sampling. There are numerous methods for sampling

Table 19.1 Requirements for using integrated pest management (IPM)	
1.	Pest manager knowledgeable about the system and management strategies
2.	Information available about the system being protected, e.g., major pests and when they are active
3.	Monitoring the numbers and state of the ecosystem elements, e.g., pest, weather, resource being protected, natural enemies of the pest
4.	Economic injury levels for the system
5.	Types of control methods
6.	Agents and materials to be applied for control, their availability and efficacy

Flint & van den Bosch, 1981.

pest populations and these differ based on the pest and the resource it is affecting. For agricultural field crops, a sampling unit could be a leaf or a bud or a specific amount of soil at the bases of plants. For example, to sample tomato fruitworm (*Helicoverpa zea*) eggs, the leaf below the highest open flower is checked because that is where most eggs are laid by the female moths. Traps baited with sex pheromones, the chemicals used by insects for communication between sexes of the same species, are often used to detect and quantify densities of moth species. Quantifying the areas on individual plants that display symptoms of infection or counting the numbers of diseased plants are used to monitor plant pathogens. Weeds per unit area can be counted to quantify weed populations. The number of samples necessary to arrive at an estimate of the pest population density can be precisely determined for each system based on desired levels of accuracy. During the time that pests are active, sampling is often done on a regular basis, with the sampling frequency dependent on the ability of the pest to increase rapidly to damaging levels. Faster growing populations require more frequent sampling.

Another critical component of IPM is knowledge of the threshold pest density above which damage will lead to economic losses (see Fig. 2.1). The concepts of IPM and economic injury levels were originated by entomologists concerned with problems caused in crops by arthropods. However, threshold levels for damaging densities due to plant pathogens and thresholds for densities of weeds have also been developed (Higley & Pedigo, 1996). This concept is not as easy to adapt to other types of pests, such as “nuisance” pests, for example fire ants living in your backyard, because it is difficult to assign a dollar value to the damage they cause.

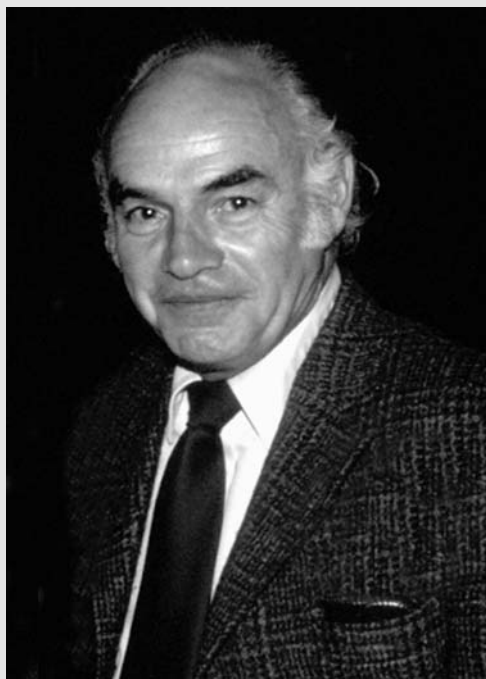
The idea of managing pest populations was first proposed in 1959 by Stern and associates in California, using the name “integrated control.” With its equitable climate, California is the state in the USA with the highest agricultural productivity. Chemical pesticides were a boon to agriculture in California and had been embraced wholeheartedly by growers after the advent of DDT. Pesticides were often applied on a schedule without checking whether the pests they were intended to control were present, let alone abundant enough to cause damage. This alarmed entomologists, who saw the resulting side-effects from excessive pesticide use (see Chapter 1) (Box 19.1).

When IPM first began to be adopted, the first stage of the transition from scheduled chemical pesticide applications was to monitor pest populations to determine whether pest densities were above damaging levels and to only use chemical pesticides when needed. This was a big change for growers but these initial changes did not incorporate all of the aspects of IPM that researchers were suggesting. With time, as researchers demonstrated that alternative control strategies could be used either along with or instead of chemical pesticides, growers began to trust this new integrated approach. While the concept of IPM is well known to many, it is used to different extents by different users and according to different systems and circumstances.

Box 19.1 Robert van den Bosch and The Pesticide Conspiracy (Hoy, 1993)

After World War II, as environmentalists became interested in preventing the non-target effects of broad spectrum synthetic chemical pesticides on the environment, entomologists were also learning that such large-scale use of chemical pesticides resulted in significant side-effects diminishing their effectiveness for control. Robert van den Bosch was born in California in 1922. After fighting in the South Pacific during World War II, he followed his interests and studied insects at the University of California, Berkeley, receiving a Ph.D. in 1950. "Van", as he was called, then spent most of his career working in the University of California system. He became involved in numerous studies involving arthropod pests in the diverse crops raised in California, but always his studies centered around biological control.

The spotted alfalfa aphid (*Therioaphis maculata*) was first detected in southern California in 1954 and it spread rapidly within a year, causing great economic losses. Growers used three organophosphate insecticides for control but soon realized that there were problems with this approach. The aphids quickly developed resistance and, with the natural enemy populations decimated by the insecticides, aphid populations resurged. A team of researchers including van den Bosch developed recommendations for growers that included use of chemicals while preserving exotic natural enemies that had been introduced. The program they developed would cost growers less than chemical pesticides alone and was more reliable. The group conducting this research was among the first to suggest such an integration of control strategies.



Robert van den Bosch. (Photo courtesy of James Carey.)

van den Bosch proceeded to conduct numerous studies to develop programs where the activity of natural enemies was used to control arthropods. He was not against all use of chemical pesticides but wanted to see them used wisely. He became frustrated because growers were not adopting recommendations for integrated control strategies. Any “new” insecticides were accepted by many growers without reservation and the pesticide treadmill would re-emerge. Van did not take this rebuff lightly, as might be the response of more bookish scientists. Along with papers presenting results from his scientific studies, Van wrote many articles on use and misuse of pesticides, including a book called *The Pesticide Conspiracy* (1978). He was often quite forthright, using picturesque language to catch the attention of pesticide proponents and the media. During public debates with pesticide proponents, Van was known to even eat a spoonful of pesticide to prove that his main issues were not with regard to immediate toxicity to humans. Van called pesticide proponents members of the “pesticide mafia” because they refused to take broader views of control options. At that time, growers often accepted control suggestions only from pesticide companies and van den Bosch saw this as “simply merchandise hustling in its most freewheeling mode.” van den Bosch’s pugnacious stance and caustic remarks were most certainly fueled by the slow adoption of integrated pest management by agriculture. He was among those entomologists suggesting the integration of approaches in 1959 (Stern *et al.*, 1959), although it took many more years before a more integrated control approach began to be accepted by some growers. Today, the legacy of van den Bosch’s activism can be seen in the continuing and growing diversification and adoption of natural enemies for pest control.

Today, adoption of IPM has differing levels that can be seen as four tiers related to varying degrees of reliance on pesticides versus natural enemies. The different tiers can be interpreted as differing levels of utilization of natural enemies ranging from complete reliance on pesticides to biointensive IPM (Table 19.2). An example of biointensive IPM would be control of flies in dairies in the northeastern USA by integrating sanitation and use of insecticides with a primary focus on use of parasitic wasps (Box 19.2).

When pest control is necessary, the alternative control strategies that can be used as part of an IPM system are many and varied. Numerous authors have presented these alternative controls using different groupings and terminology (e.g., Office of Technology Assessment, 1995; National Research Council, 1996; Nordlund, 1996). Figure 19.1 illustrates the relationship between biological control and other pest management strategies as envisioned by Eilenberg and colleagues. (2001). IPM strategies are then based on integration of a diversity of control tactics, only one of which is biological control. However, users of IPM often see biological controls as a cornerstone among these alternative controls.

19.2.1 Mechanical, physical and cultural control

Mechanical and physical controls include direct or indirect (non-chemical) methods for destroying pests or making the environment

Table 19.2 Different levels of use of natural enemies for controlling pests with IPM

IPM Type	Treatment thresholds
No IPM	Pesticide treatments based on calendar, crop stage, or pest detection but not pest quantification
Low level IPM	Pesticides applied according to thresholds associated with monitoring and timed to minimize impacts on natural enemies and beneficials
Medium level IPM	Thresholds for pesticide treatments adjusted to preserve natural enemy populations
Biointensive IPM	Development of thresholds for releasing natural enemies as alternatives to chemical pesticides

After Benbrook, 1996.

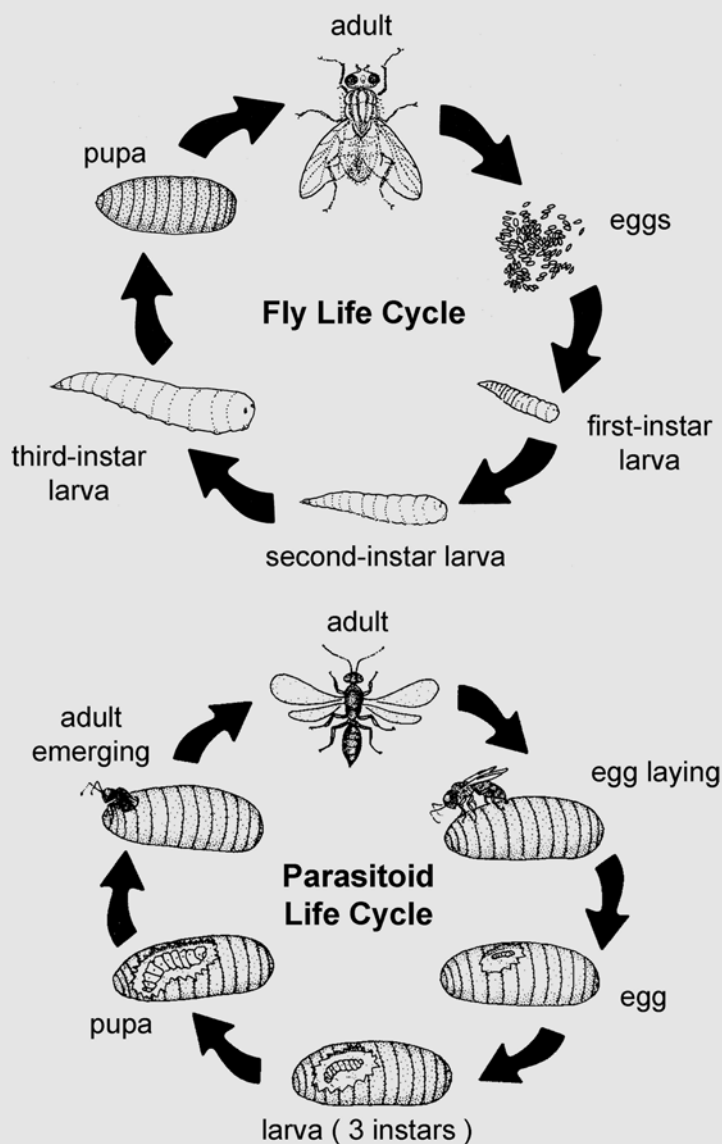
Box 19.2 | Cows without flies

Houseflies (*Musca domestica*) and stable flies (*Stomoxys calcitrans*) are common nuisances at dairies. The immature stages of these flies, maggots, develop quickly in the warm cow manure. After the advent of DDT, many dairy farmers used synthetic chemical pesticides as their only means for fly management. This practice of course resulted in development of insecticide resistance, with resistance actually found first in houseflies. Dairy farmers were probably not well aware that along with developing insecticide-resistant flies, they were killing the natural enemies that previously had been present, resulting in fly populations that were completely out of control.

An assessment of the levels of resistance to insecticides among flies in New York State dairies in 1987 suggested that the usefulness of registered pesticides for fly control was extremely limited. Don Rutz and his colleagues decided that there really must be some natural enemies of flies lurking somewhere in the environment. Once they set about it, 10 species of small parasitoids were found (Rutz & Watson, 1998). The most common was *Muscidifurax raptor*, a dark, shiny species of wasp, with adults about 1–2 mm long, that lays eggs after drilling into fly pupae with its ovipositor. *M. raptor* had the added trait that it was the only species that was abundant in both indoor and outdoor fly-breeding microhabitats.

Researchers needed to develop an integrated pest management strategy that did not rely principally on pesticides, since the registered pesticides were largely ineffective. As is typical of IPM programs, a system was developed with several different types of controls that could be used simultaneously and would complement each other. First, manure had to be removed frequently because *M. raptor* could not respond quickly enough if fly populations were huge. If fly populations were exceedingly high, a pyrethrin insecticide was applied to spaces with abundant flies to knock down the adults. However, central to this program were the inundative applications of *M. raptor*. *M. raptor* were mass-produced by a regional insectary and 200 parasitized fly pupae were purchased per cow in the herd. To release parasitoids, cheesecloth bags containing parasitized fly pupae were hung in dairy barns. Releases were made weekly for approximately 4 months during the spring and summer. Soon after releases, the wasps emerged and flew to search for unparasitized fly pupae on the top of the manure and along the edges of walls. Additional

attributes of this natural enemy are that it can search for hosts in areas that sprays cannot easily reach and it can kill pest stages that would not be susceptible to pesticides, the well-defended, thick-walled fly pupa.



Life cycles of the house fly, *Musca domestica*, and its pupal parasitoid, *Muscidifurax raptor* (Courtesy of Novartis).

For those farms where this IPM program was used, fly populations were cut in half and 80% fewer insecticide treatments were needed. In summary, this IPM program reduced the costs of pest control for the farms, lowered amounts of insecticides that had to be used, and reduced the development of resistance in flies by using lower amounts of insecticides. Last, but certainly not least, the farm was a more pleasant place to work with fewer flies buzzing around and, having fewer flies was also greatly appreciated by neighboring homes and businesses.

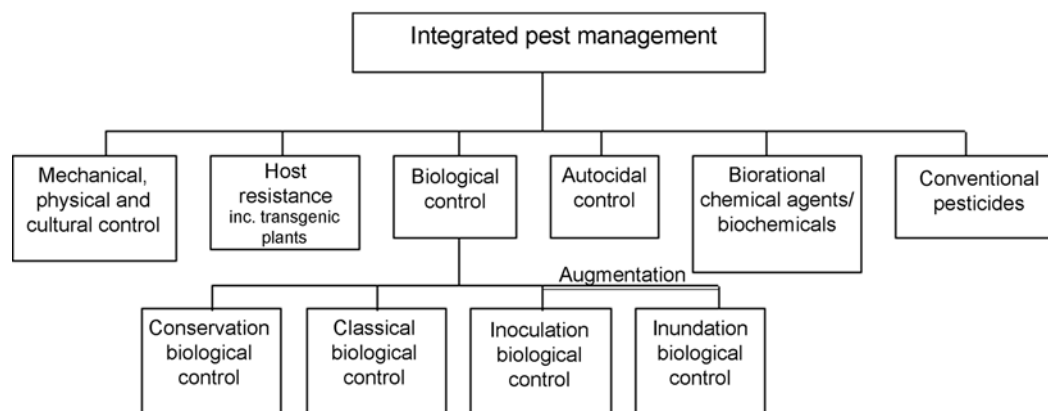


Fig. 19.1 Relationship between biological control and other strategies used for integrated pest management. The order of boxes as shown draws parallels between related strategies, such as cultural control and conservation biological control. (Eilenberg *et al.*, 2001.)

unsuitable for their entry, dispersal, survival, or reproduction. Mechanical and physical controls can be differentiated from cultural controls in that the actions taken are specifically for pest control purposes and are not merely extensions of management practices. An example would be use of metal barriers around building foundations to deter establishment of eastern subterranean termite (*Reticulotermes flavipes*) colonies. Mechanical and physical controls are often very specialized and can require considerable outlays of resources.

Cultural control involves modification of management practices to make the environment less favorable for pest reproduction, dispersal, and survival. The emphasis with this technique is on controlling the pest using tactics such as improved sanitation, crop rotation, cultivation, trap crops, or adjustment of planting dates. Crop rotation, in particular, has been used extensively for controlling plant pathogens and plant parasitic nematodes. If fields are replanted with the same crop, plant pathogens and plant parasitic nematodes that attack that crop are already present, sometimes at high levels. Rotating crops of course avoids the big problems in store if the same crop plant is planted in that same field the next year.

Conservation biological control might use the same types of techniques as cultural control, involving altering the environment, but for conservation biological control the goal is increasing the natural enemy populations while the goal of cultural control would be decreasing the pest population. Thus, the same changes to the environment could yield both conservation biological control and cultural control if, for example, a trap crop planted near a primary crop lures the pest out of the primary crop (cultural control) and flowers produced by the trap crop provide nectar for parasitic wasps that would then remain in the area and live longer to attack more pests in the crop (conservation biological control).

19.2.2 Host resistance

This strategy was developed based on the fact that different genotypes of plants can vary significantly in their acceptability to pests and in the damage caused by pests. For centuries, humans have selected the most hardy and most productive strains of crop plants to propagate

and grow. In fact, most efforts in plant breeding have focused on increasing yields of crops and not on increasing resistance to pests. Significant reductions in pest damage to plants in agriculture and landscapes can be attributed to the efforts over the past few decades of plant breeders who have shifted their focus to developing pest-resistant plant cultivars.

One kind of resistance is inducible; plants can be induced by activity of pests to produce substances that deter the pest. An example would be the antimicrobial phytoalexins such as pisatin produced in pea plants, to inhibit development of plant pathogens. Alternatively, some plants have characters that are expressed constantly to resist pests. Such constant, or constitutive, characteristics can include (a) chemical or morphological features that deter pests (antixenosis), or (b) constituents of the plant that disrupt normal growth, development or reproduction of the pest, such as toxins or digestibility reducing factors (antibiosis). Alternatively, some plant genotypes are tolerant to damage by the pests, using mechanisms such as regrowing damaged tissues or producing additional branches to compensate for damage (Kogan, 1994).

Recent advances in genetic engineering have greatly enhanced the possibilities in the area of host plant resistance by enabling the transfer of genes between widely unrelated organisms to confer resistance to pests. Of particular significance, numerous crop plants have been genetically engineered to either produce the toxins found in Bt for control of insect pests or to be resistant to the active chemical in the herbicide glyphosate. This technology has been eagerly adopted by growers in the USA but at this time has not been well accepted in European countries. A number of important issues remain unresolved, such as potential transfer of engineered genes to closely related weedy species, appropriate marketing and regulation, and impact on non-target organisms. Perhaps the greatest long-term challenge for transgenic crops is the potential ability of pests to evolve resistance under such strong selection pressure, rendering these engineered varieties useless. Pests would then also be resistant to Bt sprays, a mainstay for many growers producing organic crops who have few alternatives for pest control. In response, intensive studies are being conducted to develop methods to prevent development of resistance to Bt, and US growers planting Bt-engineered crops sign contracts stating that they will use resistance-preventing practices such as planting Bt-free refuges (see Chapter 10).

19.2.3 Autocidal control

Using this approach, individuals of the pest species are genetically altered and then released into the pest population. These individuals carry genes that interfere with reproduction or impact of the pest. This strategy is principally aimed at controlling arthropods and its principal use today is in release of sterile males for insect control. Males of the pest insect are made sterile, often by irradiation. Following release in large numbers, they compete with fertile males

for female mates, thereby reducing the number of matings that successfully produce offspring. The result is a decrease in the size of the pest population. This strategy has been particularly successful for control of the screwworm fly (*Cochliomyia hominivorax*), a serious pest of livestock. These flies lay their eggs in wounds and feeding by the maggots enlarges the wounds, which then attracts more flies to lay eggs. Large-scale releases of sterile males started in the 1950s in the southern USA. As screwworms became eliminated in areas progressively further south, the zones for fly release moved south accordingly. This pest has now been eliminated from the USA and northern Central America. By 1996, 185 million sterile flies were still being produced each week for release in Nicaragua and Honduras at a cost of US\$12 million per year (Pedigo, 1996).

19.2.4 Biorational chemical agents/biochemicals

There are numerous chemicals associated with pest control that have a biological origin. Perhaps the most commonly used of these are the pest behavior-modifying chemicals. Many organisms emit volatile chemical cues that evoke specific behaviors from other individuals of the same or a different species. Pheromones are one category of these chemicals that currently have application in pest management. Farmers, foresters, homeowners, and government agencies all rely on commercially produced pheromone products. Although the primary use of most pheromones is monitoring pest abundance and distribution, some are sold commercially for pest control. Mate-attraction pheromones are now used in pest lures or in traps laced with insecticides or microbial pesticides. The pheromone-based control method in greatest use is widespread application of pheromones to disrupt a pest's ability to find a mate, thereby reducing successful reproduction by the pest. This method, called mating disruption, is presently being used very successfully to slow the westward spread of gypsy moth in the USA.

Insect growth regulators (IGRs) are quite extensively used to control arthropods. IGRs are naturally occurring hormones or similar synthesized compounds that influence insect growth. Insects repeatedly shed and then form a new outer layer as they grow, in a process called molting. IGRs kill insects by affecting growth processes, in particular interfering with the molting process. These insecticides have low toxicity to mammals, but some IGRs affect crabs, shrimp, and other non-target invertebrates that molt. Concerns about non-target impacts on these other species, some of which are economically important, have led to stringent restrictions on allowed uses of IGRs. IGRs are now being examined with renewed interest for use in environments where such non-target impacts are highly unlikely, such as in homes or grain storage elevators. More specific IGRs might be developed for pests affecting high-value resources; however, no species-specific IGRs are presently on the market.

"Botanicals" are chemicals derived from plants that are used in the same way as conventional pesticides and are usually naturally

occurring. Examples include pyrethroids originally from chrysanthemum flowers and nicotine from tobacco. Naturally occurring botanicals enjoy popularity among organic farmers and gardeners because they are derived from “natural” sources. However, scientists believe that many botanicals are no safer as a group than synthetic chemicals and can pose the same questions of mammalian toxicity, carcinogenicity, and environmental impact.

19.2.5 Chemical pesticides

There is an immense literature on synthetic chemical pesticides and only a few aspects relevant to pest management and biological control are mentioned here. Companies producing synthetic chemical pesticides are continually working on discovering new pesticidal chemicals because pests continually become resistant to the pesticides already in use. There is a trend toward discovering chemicals with pesticidal properties that affect fewer species, to avoid problems due to non-target effects, although the trade-off for industry is that their market for each product is smaller. There is also a trend toward developing less-persistent synthetic chemical pesticides to avoid issues regarding long-lived pesticide residues. This results in the need for more frequent applications, which can add expense for the grower.

19.3 Adding an ecological emphasis to pest management

Although IPM was developed to decrease reliance on chemical pesticides, chemical pesticides remain the major type of pest control used worldwide today. Among the scientific community and many members of the public, there remains the desire to place more emphasis on increasing the use of natural enemies. Scientists have presented the challenge that even following an IPM paradigm, the result is still often short-term single-technology intervention for pest control. In many cases, often only one type of biological control or host plant resistance is used to replace chemical pesticides (e.g., Lewis *et al.*, 1997; Thomas, 1999). These arguments state that such therapeutic approaches are only treating the symptoms with the fastest-acting remedy possible, searching for the one so-called “silver bullet” that will solve the problem. Scientists argue that we should remember ecological principles and the natural balance of systems that demonstrate that such single-technology, therapeutic interventions might readily be countered by responses that would neutralize their effectiveness.

In recent years, scientists instead have suggested a more truly integrative approach that simultaneously draws on numerous methods to prevent pest problems that occur, instead of treating pests only when they become abundant. Such a “total system approach” would provide a long-term solution, a sustainable solution. This approach requires a total change in how pest problems are viewed. Instead of asking how to control one specific pest, we would be asking why the pest is a pest. Such a view would identify weaknesses in ecosystems

themselves or in how systems are managed that allow species to become pestiferous. Like IPM, the goal would not be to eliminate the pest but to bring pest densities within acceptable limits. This approach toward sustainability may be more complicated and certainly will require an in-depth understanding of the ecological interactions in each system being managed. Because a “systems” approach is being suggested, this will require scientists from numerous disciplines to work together. For example, use of non-crop plant species as cover crops is being investigated with the goal of providing habitat and food for above-ground natural enemies or controlling soil-borne plant pathogens or plant parasitic nematodes through exposure to deleterious root exudates. These same cover crops could also be effective in reducing erosion, improving soil organic matter, and providing nitrogen for subsequent crops (National Research Council, 1996). In this scenario, scientists knowledgeable about insects, plant pathogens, soils, and crops would need to work together to help develop a sustainable approach.

Specifically relative to biological control, a sustainable approach to pest management would require knowing the resident natural enemies and those factors enhancing their abundance and activity. With this knowledge would come the ability to manipulate these natural enemies, not only for short-term interventions but preferably for a long-term, sustainable solution. Inundative and inoculative strategies are more typical of short-term interventions, while long-term sustainable approaches might depend more on classical biological control and/or conservation. In particular, sustainable agriculture is aimed toward making use of resident natural enemies and enhancing their effectiveness by providing habitats and food for their survival and increase.

Understanding the interactions within a system is critical to adopting this type of strategy. The fungal pathogen *Metarhizium anisopliae* var. *acridum* being used against African locusts can be considered a rather slow-acting biopesticide for killing the target pests. However, taking the biology and ecology of the system into account adds a different perspective: (1) fungal spores produced from the bodies of the first group of locusts that die can infect a second set of locusts and a second application of the fungus is thus not needed; (2) soon after infection, feeding by locusts decreases dramatically so that damage declines long before locusts are dead; (3) infected locusts are more readily eaten by predators thus potentially increasing the predator population, and (4) periods of cloudy weather and rain will lead to higher levels of infection and more transmission of the pathogen (Thomas, 1999). This in-depth knowledge of the pathogen and ecosystem helps with understanding the utility of this pathogen by taking into account the long-term effects of the fungus and not only its immediate effects.

Matt Thomas (1999) investigated the potential for integrating plant breeding for resistance with use of natural enemies against arthropod pests both control tactics that are well known. A literature review of interactions between these strategies demonstrated that in some cases

using partially effective natural enemies along with partially resistant plants can lead to total control. However, knowledge of particular systems is critical because plant characteristics can also deter some natural enemies.

Will a sustainable approach work? As one example, an experimental farm in the Netherlands has used multidisciplinary approaches to farming, following the basic principles of sustainability. Over a 15-year period, pesticide use on the farm was reduced over 90% and, while yields were lower than commercial farms, the reduction in pesticides using “alternative” methods meant that profits were equivalent (Wijnands & Kroonen-Backbier, 1993).

19.4 | Biological control in use today

As you have learned, when we say biological control, we are talking about a diversity of strategies that are used in different contexts. But where and when are they actually being used today?

19.4.1 | Classical biological control

Classical biological control has been used extensively around the world but especially in countries where early programs yielded successes, and this certainly includes the USA. This strategy is often “stand alone” and can require several years after an introduction before the full control potential of the natural enemy is evident. Several relatively recent programs have demonstrated the same excellent results that fuel continued efforts with this strategy. Ash whitefly was introduced to California in 1988 and soon spread to 28 counties in that state as well as to Arizona and New Mexico. This whitefly was attacking ash trees, which comprise 17% of urban street trees in that region. Within two years of releasing biological control agents, the whitefly was under complete control (Fig. 3.1a), resulting in net savings of over US\$200 million (Office of Technology Assessment, US Congress, 1995). Control of the prolific floating weed water hyacinth in the United States, Australia, and the Sudan using several phytophagous insect species provides a relatively recent example of successful classical biological control of weeds (Fig. 14.1) (Goeden & Andrés, 1999).

Classical biological control programs have been criticized because their success rate is low. The counter argument has always been that cost was low so even if the program was not successful, it had not cost much. In recent years, researchers have placed more focus on understanding why some programs are unsuccessful. Based on their findings, with more effort, i.e., more funding and longer-term programs, there is now a greater probability of success but, on the other hand, classical biological control will require more of an investment (see Chapter 3).

With the increasing numbers of invasive species, there is a growing need for controlling exotic pests. While some invasives threaten

production of food and fiber, invasive species also comprise a great threat to our naturally occurring biotic diversity. Historically, classical biological control has been a key method for controlling invasive species, in some cases with spectacular results. Because classical biological control agents themselves can be thought of as invasive species, care must be taken to purposefully introduce only host-specific species, usually those with no endemics in the area of introduction that would potentially be significant hosts. Increased scrutiny regarding host specificity of natural enemies being introduced for classical biological control has been initiated to respond to requests by an environmentally aware public regarding use of this approach. Importantly, the risk of not being able to control a pest must be weighed against the risk of introducing a classical biological control agent with known host specificity.

As you have read, while some criticize classical biological control as too risky, there are certainly situations where this strategy holds the greatest promise. For example, the paperbark tea tree, *Melaleuca quinquenervia*, from Australia was introduced to Florida as an ornamental but has spread to the unique and fragile Everglades in southern Florida. This is a famous extensive wetland, sometimes referred to as the “river of grass” due to its lack of trees. This invasive tree is spreading very quickly and now covers more than hundreds of thousands of acres of the Everglades, displacing the native vegetation. The area now covered by *Melaleuca* is too great for manual removal, herbicide applications over this huge area are not really feasible, and fires to kill *Melaleuca* only result in release of copious seeds that quickly sprout to establish more plants. Classical biological control seems the best alternative and it is hoped that an effective agent can be found before this native ecosystem is altered irreversibly. This is a perfect situation to illustrate why it has been stated that classical biological control is “risky but necessary” (Thomas & Willis, 1998). Increased study of individual agents intended for classical biological control, along with overseeing which species are released, will minimize the risk in this approach.

19.4.2 Augmentation: inundative and inoculative biological control

Inundative, rather than inoculative, biological control has certainly been the major focus of augmentative releases. The emphasis on inundation makes this method for using natural enemies similar to using synthetic chemical pesticides. However, biopesticides are often viewed with skepticism by industry and crop protection specialists, who still rely principally on synthetic chemical pesticides. In fact, sales of biopesticides represent less than 1% of the global market for agrochemical crop protection and a large share of this is due to Bt (Lisansky, 1997). Biopesticides are primarily considered when chemical pesticides are (1) not available, often due to pest resistance, small market size, or because use of a pesticide has been discontinued, (2) unacceptable, as in environmentally sensitive habitats or where

human contact is high, or (3) too expensive relative to the value of the resource being protected, as in rangeland. While biopesticides are often more expensive than chemical pesticides, if their effect is more sustained, the total cost could potentially be lower because multiple applications would not be necessary.

While augmentative biological control strategies have not yet been used extensively for control of major pests in major crops, they can provide excellent control in more specialized systems, often protecting higher value resources (Gelernter & Lomer, 2000). A major challenge has been maintaining a sufficiently large market for production of these products aimed for “niche” applications. In the USA, a “niche” application would be any use other than on the major crops for which larger industry is principally interested in developing controls. There must be continuity in production of the natural enemy by industry for these products to come into regular use. Production has been a problem with microbial natural enemies to combat arthropods and weeds and some products for control of plant pathogens in developed countries, where the costs of production are higher, including massive registration costs. In developing countries where production costs can be lower and registration often doesn’t cripple getting these products to market, microbial natural enemies for use against arthropods have been produced and also used, in some cases for many years.

That being said, there are still numerous products available for augmentative use. Due to need and desire for use of an augmentative-type strategy, at least 125 species of both macroorganisms and microorganisms are presently available commercially to control arthropod pests in greenhouses (van Lenteren, 2000). Pathogens for control of numerous insect pests are variously available in different countries around the world. Eighty products (based on approximately 29 species of bacteria, fungi, and one virus) with biological control activity due to living microorganisms are available for control of plant pathogens (Whipps & Davies, 2000). A few species of phytophagous arthropods and plant pathogens are commercially available for augmentative releases against weeds.

An exceptional example of use of augmentative strategies can be found in greenhouses, especially in Europe. This use is due in part to the diversity of natural enemies that is available and the fact that they are affordable relative to the value of the crop. In Denmark, augmentative biological control is a major pest control strategy being used, and governmental support has facilitated the adoption of this strategy (Box 19.3).

Although use of natural enemies in European greenhouses is spectacular, outstanding examples of augmentative biological control are found in other systems as well. In 1995, it was estimated that the predatory mite *Phytoseiulus persimilis* was released to control two-spotted spider mites on 50–70% of the land on which strawberries were produced in California (Office of Technology Assessment, US Congress, 1995). This use really first developed in 1987, when

Box 19.3 | Augmentative biological control in Denmark

During the late 1980s, biological control became the dominant method of pest control for all greenhouse vegetables in Denmark and, as of 1999, it is also used to protect 30–35% of the greenhouse area producing ornamentals. This high usage in ornamentals is particularly interesting because little damage is tolerated on these crops. The major factors allowing use of biological control in ornamentals included (1) use of the fungal pathogen *Verticillium lecanii* on cuttings so that when new cuttings were started, they were free of insect pests (in Danish greenhouses, pests are mainly introduced with cuttings), and (2) use of the “keep-down” or “blind release” strategy of regular releases whether pests were seen or not (Eilenberg *et al.*, 2000).

Denmark is a very environmentally conscious country and the government took an active role in promoting use of biological control. Since the 1970s, the number of commercially available natural enemies has increased. In 1986, 29 active ingredients in pesticides were banned in Denmark so that many chemical pesticides could no longer be used. By 2000 (Eilenberg *et al.*, 2000), 41 species of macro- and micro-beneficials were available for use in glasshouses, with 20 of these receiving the most use. An important encouragement toward adoption of biological control by growers has been the research and advisory efforts by the Danish Institute of Agricultural Sciences. In fact, as biological control began to be adopted, this institute was the sole supplier of the whitefly-attacking parasitoid *Encarsia formosa* and two predatory mites. Outreach by this institute ensured that growers as well as the general public were aware of these changes in pest control. Companies in Denmark marketing biological control agents helped with adoption by providing advice along with natural enemies. The Ministry for the Environment also supported biological control through providing grants, and fees paid for the use of chemical pesticides were used to support basic and environmental studies toward development of biological control.

the widely used pesticide Plictran was removed from the market by federal regulation. Other alternative controls were not available and growers adopted use of these natural enemies instead.

Of course, the bacterium Bt is the most widely used microbial pesticide in the USA as well as worldwide. It forms the backbone of federal programs for control of the forest defoliator gypsy moth in the USA, although other microbial natural enemies also aid in control. In addition, Bt is used extensively against other caterpillar pests and Colorado potato beetle, and for controlling mosquitoes.

As one representative example, black vine weevil (*Otiorhynchus sulcatus*) is successfully controlled in cranberry bogs using entomopathogenic nematodes. Growers turned to nematodes because this difficult pest lives in the soil, it was susceptible to these nematodes, the nematodes were environmentally safe, this was a high value crop, and there were very few alternatives for controlling this cryptic pest.

Among the biopesticides for control of plant pathogens, a large share focus on soilborne pathogens and have been developed for greenhouse crops. The controlled environment, high-value crops and

limited numbers of registered pesticides make greenhouses a niche market needing alternative methods for pathogen control. Products based on several bacterial and fungal species (*Streptomyces*, *Bacillus*, *Coniothyrium minitans*, *Gliocladium*, *Trichoderma*, and non-pathogenic *Fusarium*) are used against several soilborne pathogens (*Sclerotinia*, *Pythium*, *Rhizoctonia* and *Fusarium*). The need for these products and their effectiveness leads to predictions that biological control of plant diseases in greenhouses could predominate over use of chemical pesticides in the United Kingdom, just as biological controls against insect pests predominate over chemical pesticides in the UK (Paulitz & Bélanger, 2001).

19.4.3 Conservation biological control

Conservation biological control has been considered the least-well-studied type of biological control (Ehler, 1998), although in the last decade several edited books have brought attention to this strategy. For classical biological control, numerous very useful databases have been maintained documenting releases and successes. For augmentative biological control, sales figures can provide some objective measure of use. It is more difficult to document the results of conservation biological control programs. As stated earlier, there are two major types of control used within this strategy, conservation of natural enemies and enhancement of natural enemies. In fact, decreasing pesticide use to preserve natural enemies can be readily quantified as savings in pesticides not purchased and applied. Reduced pesticide-induced mortality of natural enemies has aided IPM programs in rice and apples (Gurr *et al.*, 2000b). Results from the diverse methods for enhancing natural enemy populations are not as easily quantified. To date, habitat manipulation to enhance natural enemies is being implemented operationally in very few systems. Use of beetle banks in cereals in the UK is well established. Perhaps the example with the largest “real world” usage and success is found in use of the groundcover *Ageratum conyzoides*, as well as other weedy ground covers in 135,000 hectares of citrus in China (Liang & Huang, 1994). These various ground covers are beneficial for natural enemies of citrus red mite, in part by providing pollen, an alternate food that maintains populations of predatory mites. However, the majority of work on habitat manipulation to enhance natural enemies is still at the research stage.

Conservation biological control is generally not considered a “stand alone” type of control strategy. Thus, emphasis on use of conservation and enhancement of existing natural enemy populations as part of an IPM program or an ecologically based, sustainable approach to agriculture seems the most appropriate implementation for this strategy.

19.4.4 Use of natural enemies in IPM systems

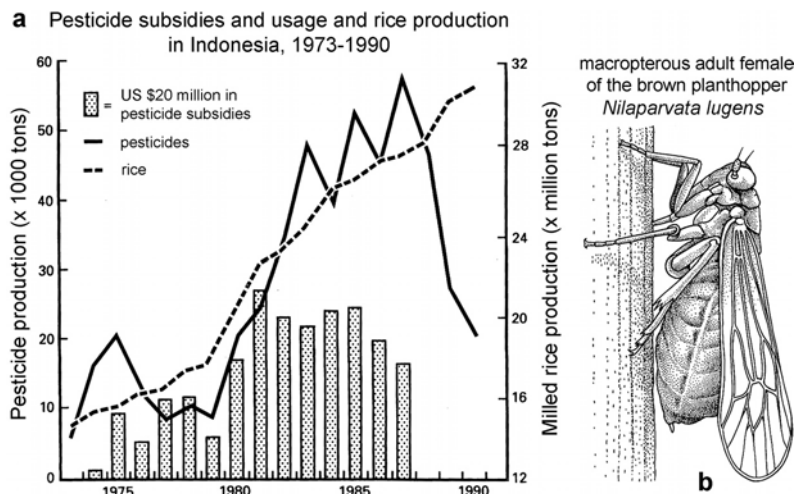
Use of natural enemies has been a central theme in IPM, especially in IPM programs that take a more biointensive approach (see Table 19.2).

Recent publications on IPM provide many examples of integration of control tactics that include use of natural enemies. An excellent example is use of parasitic wasps to control flies in dairies in north-eastern USA, resulting in decreased pesticide use as well as decreased fly populations (see Box 19.2).

While the previous example pertains to individual dairy farmers making decisions about fly control, IPM has also been adopted on much larger scales. Campbell Soup Company in the USA is a major producer and user of vegetables and other agricultural products, although its primary crop is tomato, grown in numerous states as well as Mexico (National Research Council, 1996). Campbell recognized public concerns about pesticide use and pesticide residues in foods. They encouraged their growers to adopt an IPM strategy based on sampling, cultural practices to minimize weeds, and disease and pest treatment only when necessary with use of ecologically based control methods when possible. One major concern was tomato-pathogenic geminiviruses that cause loss of tomatoes and are vectored by whiteflies. Fields that were at higher risk for whiteflies were only planted later in the season, after whitefly populations had decreased. Second, more attention was paid to field sanitation to eliminate alternate host plants for whiteflies. Several species of caterpillars are pests of tomatoes in Mexico. Traditional control would require up to 40 applications of broad spectrum insecticides per crop, yielding detectable insecticide residues. Instead, pheromones were used for mating disruption, Bt was used against tomato pinworm (*Kiefferia lycopersicella*), and *Trichogramma* were raised and released against tomato fruitworm. Insecticides against caterpillars were reduced from 22,000 pounds in Campbell's tomato crops in Sinaloa, Mexico, in 1986–87 to 0 pounds by 1992–93 (National Research Council, 1996).

The Food and Agriculture Organization was influential in facilitating diffusion of information about IPM internationally as this management strategy was developed (Kogan, 1998). The most successful FAO-supported IPM project was developed in rice in Southeast Asia. Key to the adoption of IPM was demonstrating that outbreaks of the brown planthopper, a major pest of rice, were associated with overuse of broad-spectrum insecticides (Fig. 19.2). The farmers had been counseled to overspray as insurance against pest problems but these excessive sprays led to planthopper resistance and lost profits for the farmers. Before insecticide applications were used, the natural enemies occurring in rice paddies had generally kept brown planthopper populations below the economic injury level. The increase in problems due to brown planthopper coincided with the widespread cultivation of modern, high-yielding rice varieties along with increased use of nitrogenous fertilizers and insecticides. Until 1986, the major non-insecticidal method for control of brown planthopper was use of resistant cultivars of rice but this was not enough. With the threat of the collapse of the rice industry, 57 broad spectrum insecticides were banned in Indonesia in 1987 by presidential decree, following an outbreak of brown planthoppers. IPM was instituted by creating

Fig. 19.2 Integrated pest management of rice in Indonesia. a. Once pesticide subsidies were eliminated, insecticide use plummeted but rice production increased. b. Adult female brown planthopper, *Nilaparvata lugens* (4–5 mm long). (Illustrations by Karina H. McInnes, Gullan & Cranston, 2000.)



farmer field schools in which farmers learned about the ecology of their crop and its pests and the different control measures available. This built the trust of farmers in the pest management techniques they were using. The Indonesian government took funds from pesticide subsidies and used these to fund the farmer field schools. By the end of 1995, 35,000 trainers and 1.2 million farmers had been exposed to rice IPM through this program.

FURTHER READING

- Benbrook, C. M. *Pest Management at the Crossroads*. Yonkers, New York: Consumers Union, 1996.
- Cook, R. J. Advances in plant health management in the twentieth century. *Annual Review of Phytopathology*, **38** (2000), 95–116.
- Dent, D. *Insect Pest Management*, 2nd edn. Wallingford, UK: CABI Publishing, 2000.
- Flint, M. L. & Gouveia, P. *IPM in Practice: Principles and Methods of Integrated Pest Management*. University of California, Statewide IPM Project, Agriculture & Natural Resources Publication 3418, 2001.
- Gurr, G. & Wratten, S. (eds). *Measures of Success in Biological Control*. Dordrecht, NL: Kluwer Academic Publishers, 2000.
- Hoy, M. A. & Herzog, D. E. (eds). *Biological Control in Agricultural IPM Systems*. Orlando, FL: Academic Press, 1985.
- Kogan, M. Integrated pest management: historical perspectives and contemporary developments. *Annual Review of Entomology*, **43** (1998), 243–270.
- Metcalf, R. L. & Luckmann, W. H. (eds.). *Introduction to Insect Pest Management*, 3rd edn. New York: John Wiley & Sons, 1994.
- National Research Council (US), Committee on Pest and Pathogen Control Through Management of Biological Control Agents and Enhanced Cycles and Natural Processes. *Ecologically Based Pest Management: New Solutions for a New Century*. Washington, DC: National Academy Press, 1996.
- Office of Technology Assessment (OTA; US Congress). *Biologically Based Technologies for Pest Control*. OTA-ENV-636. Washington, DC: US Government Printing Office, 1995.

-
- Pedigo, L. R. *Entomology and Pest Management*, 4th edn. Upper Saddle River, NJ: Prentice Hall, 2002.
- Rechcigl, J. E. & Rechcigl, N. A. *Biological and Biotechnological Control of Insect Pests*. Boca Raton, FL: Lewis Publishers, 1998.
- Ruberson, J. R. (ed.). *Handbook of Pest Management*. New York: Dekker, 1999.
- Winston, M. L. *Nature Wars: People vs. Pests*. Cambridge, MA: Harvard University Press, 1997.

Glossary

Definitions in this glossary are derived from a variety of sources including Coppel & Mertins, 1977; van den Bosch *et al.*, 1982; Campbell, 1989; Tanada & Kaya, 1993; Agrios, 1997; Gullan & Cranston, 2000.

Ambushers Natural enemies using a tactic of lying in wait to attack.

Specifically for entomopathogenic nematodes, raising nearly all of their body off the substrate (called nictating) to attach to passing hosts.

Foraging behaviors are species specific.

Antagonists The term used in biological control of plant pathogens to refer to natural enemies of plant pathogens. Organisms exerting damaging effects on another, for example by production of antibiotics or through competition.

Antibiosis a. Biological control of plant disease: inhibition of one microorganism by a metabolite produced by another microorganism.

b. Plant resistance: plant characteristics that affect phytophagous insects in a negative manner (e.g., reduced fecundity, increased mortality)

Antibiotic A substance produced by a microorganism that is damaging to another at low concentrations.

Antixenosis (nonpreference) Plant characteristics that cause a phytophagous insect to leave a plant.

Arthropoda Phylum of joint-legged animals having exoskeletons, including insects, mites and spiders among others.

Augmentation Biological control strategies where natural enemies are released without the expectation of permanent establishment and autonomous regulation of the host; this term includes both inoculative and inundative biological control.

Autoecious Completing its entire life cycle on the same host as compared with species that require two different hosts.

Banker plants Plants inhabited by pest and natural enemy that are introduced to an area as a source of natural enemies. The natural enemies persist on the plant and disperse to find pests on crops; used primarily in biological control in greenhouses.

Beetle banks Banks of soil within crop fields where native vegetation is allowed to establish over time thus providing habitat, especially for predatory arthropods that disperse into the crop and control pests.

Beneficial organisms In biological control, living organisms that aid in pest control (i.e., natural enemies).

Bioherbicides Natural enemies applied to control weeds using augmentation biological control.

Biological control (or Biocontrol) The use of living organisms to suppress the population of a specific pest organism, making it less abundant or less damaging than it would otherwise be.

Biologically based pest management Managing pests using biologically based technologies when control is needed.

Biologically based technologies This umbrella term has been defined as including biological control (all strategies for use for all kinds of natural enemies except inundative use of microbes), microbial pesticides

- (microbes applied repeatedly to suppress pest populations, and genetic engineering applied to this approach), pest-modifying chemicals, genetic manipulations of pest populations, and plant immunization (OTA, 1995).
- Biopesticides** Natural enemies applied for augmentation biological control.
- Bioprotectant** Natural enemies released when the pest is not present so the pest will be prevented from causing damage. Used in plant pathology when antagonists are applied to healthy plants or seeds so that pathogens will not be able to infect.
- Biorational pest management** Pest management techniques that are based on biological and ecological interactions that reduce the negative effects of pest populations without harmful side effects to man or the environment (Nordlund, 1996). Included in this term are cultural control, host resistance, biological control, autocidal control, and biorational chemical agents.
- Biotroph** A pathogen that requires living host tissue for its nutrition; term used in plant pathology.
- Biotype** A strain of a species, morphologically indistinguishable from other members but exhibiting distinct physiological characteristics, especially with regard to its adaptation to climatic conditions.
- Blind releases** Greenhouse applications of macro-natural enemies on a regular basis, regardless of finding pests.
- Canker** An area of a stem, branch, or twig dead and sunken due to activity of a plant pathogen.
- Chronic** Occurring over a long period of time.
- Classical biological control** The intentional introduction of an exotic biological control agent for permanent establishment and long-term pest control.
- Coevolution** Evolutionary interactions between two closely associated organisms, for example a parasitoid and its phytophagous host, usually referring to changes taking place in which the evolution of a specific trait in one organism leads to reciprocal development of a trait in the second organism, directly in response to the initial trait change in the first.
- Competitive displacement** Displacement of one species by another that is competitively superior; in biological control this can occur when a superior competitor is introduced to a new area.
- Competitors** Organisms interfering with each other because they require the same limiting resource.
- Conductive soil** A soil that allows development of plant disease.
- Conidia (singular, conidium)** Asexual fungal spores formed on the ends of specialized hyphae called conidiophores.
- Conservation biological control** Modification of the environment or existing practices to protect and enhance specific natural enemies or other organisms to reduce the effects of pests.
- Constitutive (characteristics)** Something always present (opposite of inducible).
- Cross protection** Protection of a plant from disease by inoculation, often with an avirulent strain of a virus that then protects the plant from infection by more virulent strains of the same virus.
- Cruisers** Entomopathogenic nematodes that do not raise their bodies off the substrate to forage (as opposed to ambushers) and display more movement searching for hosts than ambushers. Foraging behaviors are species specific.

Damping off Mortality of seedlings near the soil surface, often evidenced by wilted and decaying seedlings lying on the soil.

Defoliation Removing leaves from a plant, often used in entomology to refer to insects feeding on woody plants.

Delayed density-dependent mortality Mortality of members of a population, with the magnitude of this effect determined by the density of the population at some time in the past.

Delta (δ) endotoxin Toxic protein occurring as a protoxin in a crystalline form within sporangia (spore-bearing cells) of *Bacillus thuringiensis*.

Density-dependent mortality Mortality of members of a population in relation to the density of the prey or host population.

Density-independent mortality Mortality of members of a population with no relation to density of the prey or host population, such as due to severe weather.

Disease Invertebrate pathology: state of not being healthy. Plant pathology: malfunctioning of host cells and tissues leading to development of external or internal reactions or alterations of a plant.

Domatia (singular, domatium) Small protected areas often located where primary veins converge on leaves in some woody plants and consisting of a depression that can be enclosed by leaf tissue or hairs.

Ecological host range The range of hosts naturally found to be utilized by a natural enemy in the field.

Ecologically based pest management Pest management based on facilitating the natural processes controlling pests but supplemented by biological-control organisms, biological-control products (genes or gene products derived from living organisms), narrow-spectrum synthetic chemicals, and resistant plants (National Research Council, 1996).

Economic injury level The density of a pest population at which the cost of control measures is justified. At these pest densities, the cost of control is equal to the loss suffered if control action is not taken.

Ecotype A specific strain of a species adapted to a certain suite of environmental conditions.

Ectoparasitoid A parasitoid that develops externally on the body of a host.

Encapsulation An immune response by arthropods where an invader is surrounded by blood cells that eventually form a capsule.

Endemic Native, originating in and limited to a geographic area.

Endoparasitoid A parasitoid that develops internally within the body of the host.

Entomopathogenic Adjective describing pathogens infecting insects.

Epidemic An unusually large number of cases of disease.

Epiphytic Occurring on the surface of a plant or plant organ without causing infection.

Epizootic An unusually large number of cases of disease in a non-human animal population, analogous to the term epidemic.

Eradication Total elimination of an organism from an area.

Eukaryote Organisms with cells containing genetic material within a nucleus. This excludes viruses, prions, bacteria, and other microorganisms lacking a nucleus.

Exotic Introduced from another geographic area; now also called invasive.

Facultative pathogen An organism usually living as a saprophyte but also having the ability to live as a pathogen.

- Facultative saprotroph** An organism usually living as a parasite but also having the ability to live as a saprophyte using dead organic matter for food.
- Formulation** Relative to microorganisms for use in biological control, this term refers to ingredients added to aid in preserving organisms, delivering them to targets, and improving their survival and activity.
- Frass** Solid excreta from insects, especially larvae.
- Functional response** Response of predators to prey in which the number of prey eaten by predators changes in response to prey density, i.e., more prey are eaten when prey density is greater.
- Gall** A swelling or overgrowth on a plant that results from stimulation by fungi, bacteria, or arthropods.
- Green manure** A growing crop plowed under to enrich the soil.
- Gregarious parasitoid** A parasitoid for which more than one individual can develop in or on one host individual.
- Guild** Group of species that use a common resource base in a similar way.
- Habitat enhancement** One major type of conservation biological control focusing on enhancing habitats to increase natural enemy populations or activity.
- Hemimetabola** Insects with body forms gradually changing at each molt, with wing buds increasing gradually to the adult state, i.e. undergoing incomplete metamorphosis.
- Hemocoel** The main body cavity of many invertebrates, including insects and mites, equivalent to a circulatory system and filled with “blood” or hemolymph.
- Herbivore** Animal feeding on living plants.
- Holometabola** Insects with body forms abruptly changing at the molt to pupa and again at the molt to adult, i.e. undergoing complete metamorphosis.
- Honeydew** Fluid containing sugars excreted from the anus of some Homoptera (e.g., aphids, scale insects, mealybugs, whiteflies).
- Host** An organism that harbors another organism such as a pathogen, parasite, or parasitoid, either internally or externally.
- Host feeding** Activity by females of some parasitoid species of piercing a host with the ovipositor and then drinking host fluids that ooze from the wound. This is not always fatal to the host.
- Host specificity** The degree of limitation in the number of different plant or animal species that can be used by organisms using other organisms as food, for example, herbivores, predators, parasites, or pathogens.
- Hyperparasite** A parasite that lives at the expense of another parasite (used with reference to both plant pathogens and insects).
- Hyperparasitoid** A parasitoid that lives at the expense of another parasitoid (used with reference to insects only).
- Hypha (plural, hyphae)** A singular, usually tubular, branch of the body of a fungus.
- Hypovirulence** Reduced virulence.
- Idiobiont** A parasitoid attacking hosts that do not develop further after parasitization.
- Induced** When a specific action occurs in response to a stimulus.
- Infection** Invertebrate pathology: entry of a pathogen into the body of a susceptible host. Plant pathology: establishment of a pathogen within a host plant.

- Infective juvenile** Entomopathogenic nematodes that disperse and infect a new host, often third stage.
- Inoculative biological control (Inoculative augmentation)** The intentional release of a living organism as a biological control agent with the expectation that it will multiply and control the pest for an extended period, but not that it will do so permanently.
- Instar** For insects, the growth stage between two successive molts.
- Intraguild predation** Predation by one member of a guild on another member of the same guild, for example two predators that feed on the same prey, when one predator eats the second.
- Introduced** Invasive, non-native.
- Inundative biological control (Inundative augmentation)** The use of living organisms to control pests when control is achieved exclusively by the organisms that have been released. Therefore, reproduction by the organisms released is not expected for control.
- Invasive** Not native to that region.
- Invertebrate** An animal without a backbone.
- Koinobiont** A parasitoid attacking hosts that continue developing after parasitization.
- Larva (plural, larvae)** An immature insect after it has emerged from the egg and before pupation; usually refers to holometabolous insects.
- Lesion** An injury or hurt, commonly used to refer to injuries to plants caused by growth of pathogens.
- Life table** A table documenting complete data on the mortality of a population through time. For insects, this is often organized as mortality by instar.
- Mating disruption** Mass application of pheromones so that mates cannot find each other.
- Melanization** The process through which invertebrates darken through deposition of melanin. This commonly occurs after an organism molts and can also occur inside the body when blood cells encapsulate an invader.
- Metamorphosis** A relatively abrupt change in body form between the end of immature development and the adult stage.
- Metapopulation** A set of local populations connected to each other through dispersal.
- Microbial control** The use of microorganisms as biological control agents. This should be considered a sub-set of biological control.
- Microbial pesticides** Microorganisms used for inoculative or inundative biological control.
- Microhabitat** A small habitat, often only micrometers in diameter, where a microorganism lives.
- Microorganism (Microbe)** A living organism visible only when using a microscope.
- Molting** The periodic process of shedding the outer covering; for arthropods this is necessary during larval growth because the hardened exoskeleton does not allow further growth.
- Monoculture** Cultivation with a single plant species.
- Monophagous** Feeding on only one species of organism for food.
- Multiple parasitism** More than one parasitoid species occurs simultaneously in or on the body of a host.
- Mutualism** Symbiotic relationship where both members of the relationship benefit from the association.

Mycetocyte A cell within insects containing symbiotic microorganisms. Such cells can be scattered throughout the body (particularly found in the fat body) or aggregated in an organ called a **mycetome**.

Mycoherbicide A fungal pathogen of weeds used for inundative or inoculative biological control.

Mycoparasite Plant pathology: term specifically meaning a fungus parasite on other fungi.

Mycorrhiza (plural, mycorrhizae) A symbiotic association between a fungus and the roots of a plant.

Native Originating in that area, part of the naturally occurring flora and fauna.

Natural enemies In a general sense, the parasites, predators, pathogens, competitors and other antagonists associated with a species of animal or plant that cause debility or mortality.

Necrotroph A microorganism feeding only on dead organic tissues; generally used in plant pathology.

New association Classical biological control term referring to situations where a natural enemy is used against a host with which it did not coevolve, i.e., the natural enemy and host did not come from the same area originally. This has also been called neoclassical biological control.

Niche The set of ecological conditions under which a species can live and eat such that it is able to reproduce and exploit similar conditions elsewhere.

Non-target organism In the context of biological control, all species that are not the pest(s) that are the focus of control programs.

Numerical response Response by predators to prey in which density of predators in a given area increases due to reproduction in relation to increasing prey density.

Nymph An immature insect without wings or with incomplete wings, usually referring to hemimetabolous insects. These immatures are usually relatively similar to adults in appearance.

Obligate pathogen A pathogen that, in nature, can only grow and reproduce in or on another living organism.

Occlusion bodies Structures produced by certain viruses, made of a protein matrix containing virions (also called polyhedral occlusion bodies).

Oligophagous Feeding on a limited number of species for food.

Outbreak (population) Explosive increase in the abundance of a species over a relatively short time period.

Oviposition Egg laying.

Ovipositor Abdominal structure of insect parasitoids used for laying eggs (oviposition); often a long tube through which eggs pass. The ovipositor can be used to lay eggs on surfaces or can be used to inject eggs into hosts or structures in which hosts live.

Parasite An organism that uses another organism for nourishment, living on or in a host that receives no benefit from the association.

Parasitoid An insect that, when immature, parasitizes another insect, killing its host, and is subsequently free-living as an adult.

Parasporal body (or parasporal crystal) In *Bacillus thuringiensis*, the crystal within the sporangium (spore-bearing cell) that contains the protoxin that will be cleaved to become the active delta (δ) endotoxin.

Pathogen A microorganism that lives as a parasite on or in a larger host organism, causing debility or mortality.

- Pest** A species that inflicts harm to humans, domesticated animals, or cultivated crops; basically, organisms that compete with humans and their needs.
- Pesticide** Any substance used for controlling, preventing, destroying, disabling, or repelling a pest, including such substances as insecticides, herbicides, and fungicides.
- Pesticide treadmill** A syndrome through which use of pesticides leads to pesticide resistance, pest resurgence, and secondary pest outbreaks which then result in increased pesticide use, only leading to repeated occurrences of these effects, resulting in increasing, ineffective, pesticide use.
- Phagostimulant** A compound that stimulates feeding.
- Pheromone** A substance secreted by one animal that causes a specific reaction when received by another animal of the same species.
- Phloem-feeding** Feeding on liquid running through the nutrient-conducting tissues of plants.
- Physiological host range** The range of hosts that a natural enemy can utilize for growth and reproduction. This differs from the ecological host range because these hosts might not be encountered by the natural enemy in nature.
- Phytoalexins** Substances produced in higher plants in response to chemical, physical, or biological stimuli, which inhibit the growth of certain microorganisms.
- Phytophagous insect** An insect that feeds on plants.
- Plasmid** An extrachromosomal piece of DNA that can replicate independently and can be transferred between organisms.
- Polar filament** When a microsporidian spore germinates, the tube that everts and extends to puncture a host cell. The cytoplasm of the microsporidian cell then flows through the polar filament and into the pierced host cell.
- Polyculture** Simultaneously growing two or more species or cultivars of plants at agronomically close distances, also called intercropping.
- Polyphagous** Feeding on a diversity of species for food.
- Population regulation** Relative to population dynamics, the control of a population; often used as return of a population to an equilibrium density as a result of density-dependent processes.
- Predator** An animal that eats more than one other animal during its life.
- Pre-emptive exclusion** Colonization of a resource by a microorganism before other microorganisms, thus excluding newcomers. These pioneer colonists can alter the habitat so later-arriving microorganisms cannot successfully compete.
- Prey** A food item of a predator.
- Primary infection** The first infection of the season by the persistent stages of a pathogen.
- Primary parasitoid** A parasitoid that develops in or on a host that is not a parasite.
- Prokaryote** Organisms whose genetic material is not contained within a nucleus. This includes the bacteria, viruses, and their relatives such as prions.
- Proovigenic** Parasitoids that emerge as adults with their full lifetime complement of mature eggs.

Protista A diverse group of eukaryotic one-celled organisms distinct from multicellular animals and plants, including protozoa, slime molds, and eukaryotic algae.

Recombinant In which segments of two DNA molecules have been exchanged. If recombination occurs, progeny from a cross between two different parents would have combinations of alleles not displayed by either parent.

Replication Reproduction, copying.

Resistance The ability of an organism to exclude or overcome, completely or partially, the effect of a deleterious agent. Often used to describe the ability of previously susceptible pests to now survive exposure to specific synthetic chemical pesticides. Also for plant/pest interactions, this term refers to the ability of plants to lessen the effects of herbivore or plant pathogen attacks.

Rhizobacteria Bacteria living in the root zone.

Rhizosphere The soil around a living plant root.

Rhizosphere competent Microorganisms surviving and remaining active around roots as the roots develop and grow.

Sampling The process of taking a small part of something as a sample to provide estimation about the whole.

Saprophyte An organism using dead organic matter as food.

Sclerotization Stiffening of the cuticle of an invertebrate by cross-linkage of protein chains. This process commonly occurs after a molt.

Scout Sample to detect pests.

Seasonal inoculative release When a natural enemy does not persist after release due to seasonal effects and is thus released inoculatively each season.

Secondary cycling In an arthropod disease, cycles of infection to death to production of inoculum and infection of another host that occur after a first cycle during one season; the first cycle is initiated by primary infections from persistent inoculum and later cycles are caused by secondary infections.

Secondary infection Infection caused by inoculum produced as a result of primary or a subsequent infection.

Secondary pest outbreak Rapid numerical response (increase through reproduction) to pest status by an organism that is typically not pestiferous. Commonly occurs after use of a broad-spectrum pesticide for control of a primary pest, resulting in mortality of the natural enemies that normally regulate populations of the secondary pest.

Secondary plant compounds Plant chemicals that function in defense against herbivores or plant pathogens.

Selection pressure Effect of any feature of the environment, such as activity of natural enemies, that leads to natural selection, or differential reproduction by different genotypes.

Sequester In the context of interactions between herbivores and secondary plant compounds: when a compound is separated from the ingested plant material and stored within the herbivore, often considered as subsequently providing defense.

Siderophore A chemical produced by an organism that binds cations, especially Fe^{+2} , and helps to transport the cations into the organism, especially occurring in environments where that cation is limiting.

- Solitary parasitoid** Parasitoid species for which only one individual can develop in or on one host individual.
- Spiracle** For arthropods, external opening of the gas exchange system which is composed of series of ever-smaller tubes called tracheae and tracheoles.
- Sporangium (plural, sporangia)** A structure containing one or more asexual spores; term used for bacteria and fungi.
- Spore** Reproductive unit of numerous microorganisms including fungi and bacteria, consisting of one or more cells; analogous to a seed of a green plant.
- Superparasitism** Situation where more parasitoid larvae occur in or on a host than can survive.
- Suppressive soil** A soil in which plant disease is reduced or absent, although the pathogen and a susceptible host are both present.
- Sustainable agriculture** Farming systems that are capable of maintaining their productivity and usefulness to society indefinitely; they should be resource-conserving, socially supportive, commercially competitive, and environmentally sound.
- Symbiosis** Living together of two or more different kinds of organisms.
- Synovigenic** Parasitoid species with adult females that mature eggs through part or all of their adult lives.
- Systemic acquired resistance (SAR)** Phenomenon occurring when a plant infected with one pathogen is more resistant to another.
- Target pest resurgence** Rapid increase of a pest population after use of a broad-spectrum pesticide that results in destruction of the natural enemies normally helping to control populations of the pest.
- Tissue specific** Restricted to specific tissues, as is characteristic of some microsporidia and viruses.
- Tolerance** In plant resistance, the ability of a host plant to withstand injury by pests.
- Transconjugate** In prokaryotes, transfer of DNA from one cell to another.
- Transgenic** Used to describe genetically modified organisms containing foreign genes inserted by means of recombinant DNA.
- Trichome** Hair-like outgrowth from the epidermal cell of a plant.
- Tritrophic interactions** Interactions affecting organisms of three trophic levels in a community.
- Triungulin** An active first instar larva of insects that usually disperses before changing into a less-dispersive stage in later larval instars.
- Vector** A bearer. An organism that transmits a disease-producing organism from one host to another.
- Vertebrate** Animal with a backbone.
- Virion** Viral DNA or RNA and the protein capsule surrounding it.
- Virulence** The relative capacity of a pathogen to cause disease.

References

- Adams, B. J. & Nguyen, K. B. (2002). Taxonomy and systematics. In *Entomopathogenic Nematology*, ed. R. Gaugler, pp. 1–33. Wallingford, UK: CABI Publishing.
- Agarwal, A. A. (2000). Mechanisms, ecological consequences and agricultural implications of tri-trophic interactions. *Current Opinion in Plant Biology*, **3**, 329–335.
- Agrios, G. N. (1997). *Plant Pathology*, 4th edn. San Diego, CA: Academic Press.
- Albajes, R., Gullino, M. L., van Lenteren, J. C. & Elad, Y. (ed.) (1999). *Integrated Pest and Disease Management in Greenhouse Crops*. Dordrecht, NL: Kluwer Academic Publishers.
- Andow, D. A. (1991). Vegetational diversity and arthropod population response. *Annual Review of Entomology*, **36**, 561–586.
- Andrews, J. H. & Harris, R. F. (1986). *r*- and *K*-selection and microbial ecology. *Advances in Microbial Ecology*, **9**, 99–147.
- Annecke, D. P., Karny, M. & Burger, W. A. (1969). Improved biological control of the prickly pear, *Opuntia megacantha* Salm-Dyck, in South Africa through use of an insecticide. *Phytophylactica*, **1**, 9–13.
- Askew, R. R. (1971). *Parasitic Insects*. London: Heinemann Educational Books.
- (1975). The organisation of chalcid-dominated parasitoid communities centred upon endophytic hosts. In *Evolutionary Strategies of Parasitoids*, ed. P. W. Price, pp. 130–153. New York: Plenum Press.
- Baker, K. F. (1987). Evolving concepts of biological control of plant pathogens. *Annual Review of Plant Pathology*, **25**, 67–85.
- Baker, K. F. & Cook, R. J. (1974). *Biological Control by Plant Pathogens*. San Francisco, CA: Freeman.
- Baker, K. F. & Griffin, G. J. (1995). Molecular strategies for biological control of fungal plant pathogens. In *Novel Approaches to Integrated Pest Management*, ed. R. Reuveni, pp. 153–182. Boca Raton, FL: CRC Press.
- Barbosa, P. (ed.) (1998). *Conservation Biological Control*. San Diego, CA: Academic Press.
- Barbosa, P., Saunders, J. A., Kemper, J., Trumbule, R., Olechno, J. & Martinat, P. (1986). Plant allelochemicals and insect parasitoids. Effects of nicotine on *Cotesia congregata* (Say) (Hymenoptera: Braconidae) and *Hyposoter annulipes* (Cresson) (Hymenoptera: Ichneumonidae). *Journal of Chemical Ecology*, **12**, 1,319–1,328.
- Barbosa, P., Gross, P. & Kemper, J. (1991). Influence of plant allelochemicals on the tobacco hornworm and its parasitoid, *Cotesia congregata*. *Ecology*, **72**, 1,567–1,575.
- Barron, G. L. (1979). Observations on predatory fungi. *Canadian Journal of Botany*, **57**, 187–193.
- Bateman, R. P. (2000). Rational pesticide use: an alternative escape from the treadmill? *Biocontrol News & Information*, **21**, 96–100.
- Baum, J. A., Johnson, T. B. & Carlton, B. C. (1999). *Bacillus thuringiensis*, natural and recombinant bioinsecticide products. In *Biopesticides, Use and Delivery*, ed. F. R. Hall & J. J. Menn, pp. 189–209. Totowa, NJ: Humana Press.
- Becker, N. (2000). Bacterial control of vector-mosquitoes and black flies. In *Entomopathogenic Bacteria: From Laboratory to Field Application*, ed. J.-F.

- Charles, A. Delécluse & C. Nielsen-LeRoux, pp. 383–398. Dordrecht, NL: Kluwer Academic Publishers.
- Bedding, R. A. (1993). Biological control of *Sirex noctilio* using the nematode *Deladenus siricidicola*. In *Nematodes and the Biological Control of Insect Pests*, ed. R. Bedding, R. Akhurst & H. Kaya, pp. 11–20. East Melbourne, Victoria, Australia: CSIRO Publications.
- Bellows, T. S. & Fisher, T. W. (ed.) (1999). *Handbook of Biological Control*. San Diego, CA: Academic Press.
- Bellows, T. S. & Headrick, D. H. (1999). Arthropods and vertebrates in biological control of plants. In *Handbook of Biological Control*, ed. T. S. Bellows & T. W. Fisher, pp. 505–516. San Diego, CA: Academic Press.
- Bellows, T. S., Paine, T. D., Gould, J. R., Bezark, L. G. & Ball, J. C. (1992). Biological control of ash whitefly: a success in progress. *California Agriculture*, **46**, 24–28.
- Benbrook, C. M. (1996). *Pest Management at the Crossroads*. Yonkers, New York: Consumers Union.
- Berenbaum, M. R. (1995). Turnabout is fair play: secondary roles for primary compounds. *Journal of Chemical Ecology*, **21**, 925–940.
- Bergelson, J. & Crawley, M. J. (1989). The theory and practice of biological control. *Comments on Modern Biology, C, Comments on Theoretical Biology*, **1**, 197–215.
- Blossey, B. & Hunt-Joshi, T. R. (2003). Belowground herbivory by insects: influence on plants and aboveground herbivores. *Annual Review of Entomology*, **48**, 521–547.
- Blossey, B., Skinner, L. C. & Taylor, J. (2001). Impact and management of purple loosestrife (*Lythrum salicaria*) in North America. *Biodiversity and Conservation*, **10**, 1,787–1,807.
- Boettner, G. H., Elkinton, J. S. & Boettner, C. J. (2000). Effects of a biological control introduction on three nontarget native species of saturniid moths. *Conservation Biology*, **14**, 1,798–1,806.
- Boucias, D. G. & Pendland, J. C. (1998). *Principles of Insect Pathology*. Boston, MA: Kluwer Academic Publishers.
- Briese, D. T. & McLaren, D. A. (1997). Community involvement in the distribution and evaluation of biological control agents: landcare and similar groups in Australia. *Biocontrol News & Information*, **18**, 39N–49N.
- Brown, G. C. (1987). Modeling. In *Epizootiology of Insect Diseases*, ed. J. R. Fuxa & Y. Tanada, pp. 43–68. New York: John Wiley & Sons.
- Bugg, R. L. & Pickett, C. H. (1998). Introduction: enhancing biological control – habitat management to promote natural enemies of agricultural pests. In *Enhancing Biological Control: Habitat Management to Promote Natural Enemies of Agricultural Pests*, ed. C. H. Pickett & R. L. Bugg, pp. 1–23. Berkeley, CA: University of California Press.
- Bull, D. L. & Menn, J. J. (1990). Strategies for managing resistance to insecticides in *Heliothis* pests of cotton. In *Managing Resistance to Agrochemicals*, ed. M. B. Green, H. M. LeBaron & W. K. Moberg, pp. 118–133. Washington, DC: American Chemical Society.
- Burdon, J. J. & Marshall, D. R. (1981). Biological control and the reproductive mode of weeds. *Journal of Applied Ecology*, **18**, 649–658.
- Burges, H. D. (ed.) (1998). *Formulation of Microbial Biopesticides*. Dordrecht, NL: Kluwer Academic Publishers.
- Butler, L., Zivkovich, C. & Sample, B. E. (1995). Richness and abundance of arthropods in the oak canopy of West Virginia's Eastern Ridge and Valley Section during a study of impact of *Bacillus thuringiensis* with

- emphasis on macrolepidoptera larvae. Bulletin, West Virginia University Agricultural and Forestry Experiment Station, **711**, 19 pp.
- Cade, W. (1975). Acoustically orienting parasitoids: fly phonotaxis to cricket song. *Science*, **190**, 1,312–1,313.
- Caltagirone, L. E. (1981). Landmark examples in classical biological control. *Annual Review of Entomology*, **26**, 213–232.
- Caltagirone, L. E., Shea, K. P. & Finney, G. L. (1964). Parasites to aid control of navel orangeworm. *California Agriculture*, **18**, 10–12.
- Campbell, R. (1989). *Biological Control of Microbial Plant Pathogens*. Cambridge: Cambridge University Press.
- Cartwright, B. & Kok, L. T. (1990). Feeding by *Cassida rubiginosa* (Coleoptera: Chrysomelidae) and the effects of defoliation on growth of musk thistles. *Journal of Entomological Science*, **25**, 538–547.
- Center, T. D., Hill, M. P., Cordo, H. & Julien, M. H. (2002). Waterhyacinth. In *Biological Control of Invasive Plants in the Eastern United States*, ed. R. Van Driesche, B. Blossey, M. Hoddle, S. Lyon & R. Reardon, pp. 41–64. USDA, Forest Service, FHTET-2002-04.
- Chandler, J. M. (1980). Assessing losses caused by weeds. In *Proceedings of the E. C. Stakman Communication Symposium*, ed. R. J. Aldrich. St. Paul, MN: Department of Plant Pathology, University of Minnesota.
- Chaney, W. E. (1998). Biological control of aphids in lettuce using in-field insectaries. In *Enhancing Biological Control: Habitat Management to Promote Natural Enemies of Agricultural Pests*, ed. C. H. Pickett & R. L. Bugg, pp. 73–83. Berkeley, CA: University of California Press.
- Charudattan, R. (1991). The mycoherbicide approach with plant pathogens. In *Microbial Control of Weeds*, ed. D. O. TeBeest, pp. 24–57. New York: Chapman & Hall.
- (2001). Biological control of weeds by means of plant pathogens: significance for integrated weed management in modern agro-ecology. *BioControl*, **46**, 229–260.
- Charudattan, R., Chandramohan, S. & Wyss, G. S. (2002). Biological control. In *Pesticides in Agriculture and the Environment*, ed. W. B. Wheeler, pp. 25–58. New York: Marcel Dekker.
- Clarke, A. R. & Walter, G. H. (1995). “Strains” and the classical biological control of insect pests. *Canadian Journal of Zoology*, **73**, 1,777–1,790.
- Coll, M. (1998). Parasitoid activity and plant species composition in intercropped systems. In *Enhancing Biological Control: Habitat Management to Promote Natural Enemies of Agricultural Pests*, ed. C. H. Pickett & R. L. Bugg, pp. 85–119. Berkeley, CA: University of California Press.
- Cook, R. J. (1993). Making greater use of introduced microorganisms for biological control of plant pathogens. *Annual Review of Phytopathology*, **31**, 53–80.
- Cook, R. J. & Baker, K. F. (1983). *The Nature and Practice of Biological Control of Plant Pathogens*. St. Paul, MN: The American Phytopathological Society.
- Coppel, H. C. & Mertins, J. W. (1977). *Biological Insect Pest Suppression*. Berlin: Springer-Verlag.
- Copping, L. G. (ed.) (2001). *The Biopesticide Manual*, 2nd edn. Farnham, UK: British Crop Protection Council.
- Costa, A. S. & Müller, G. W. (1980). Tristeza control by cross protection. *Plant Disease*, **64**, 538–541.
- Cranshaw, W., Sclar, D. C. & Cooper, D. (1996). A review of 1994 pricing and marketing by suppliers of organisms for biological control of arthropods in the United States. *Biological Control*, **6**, 291–296.

- Crawley, M. J. (1989). The successes and failures of weed biocontrol using insects. *Biocontrol News & Information*, **10**, 213–223.
- Cruz, Y. P. (1981). A sterile defender morph in a polyembryonic hymenopterous parasite. *Nature*, **294**, 446–447.
- Culver, J. J. (1919). A study of *Compsilura concinnata*, an imported tachinid parasite of the gipsy moth and the brown-tail moth. *United States Department of Agriculture Bulletin* 766.
- Dahlsten, D. L. & Garcia, R. (ed.) (1989). *Eradication of Exotic Pests*. New Haven, CT: Yale University Press.
- Darwin, C. (1859). *On the Origin of Species*. London: John Murray & Sons.
- Davies, K. G., Flynn, C. A., Laird, V. & Kerry, B. R. (1990). The life-cycle, population dynamics and host specificity of a parasite of *Heterodera avenae*, similar to *Pasteuria penetrans*. *Revue de Nématologie*, **13**, 303–309.
- Deacon, J. W. (1991). Significance of ecology in the development of biocontrol agents against soil-borne plant pathogens. *Biocontrol Science and Technology*, **1**, 5–20.
- DeBach, P. H. (1964a). *Biological Control of Insect Pests and Weeds*. New York: Reinhold Publishing Corporation.
- (1964b). The scope of biological control. In *Biological Control of Insect Pests and Weeds*, ed. P. H. DeBach, pp. 3–20. New York: Reinhold Publishing Corporation.
- DeBach, P. & Rosen, D. (1991). *Biological Control by Natural Enemies*. Cambridge: Cambridge University Press.
- DeBach, P. & Sundby, R. A. (1963). Competitive displacement between ecological homologues. *Hilgardia*, **34**, 105–166.
- DeBach, P., Rosen, D. & Kennett, C. E. (1971). Biological control of coccids by introduced natural enemies. In *Biological Control*, ed. C. B. Huffaker, pp. 165–194. New York: Plenum Press.
- De Moraes, C. M., Lewis, W. J., Paré, P. W., Alborn, H. T. & Tumlinson, J. H. (1998). Herbivore-infested plants selectively attract parasitoids. *Science*, **393**, 570–573.
- de Oliveira, M. R. V. (1998). South America. In *Insect Viruses and Pest Management*, ed. F. R. Hunter-Fujita, P. F. Entwistle, H. F. Evans & N. E. Crook, pp. 339–355. Chichester, UK: Wiley.
- Delfosse, E. S. & Cullen, J. M. (1981). New activities in biological control of weeds in Australia. II. *Echium plantagineum*: curse or salvation? *Proceedings of the Fifth International Symposium on Biological Control of Weeds, Brisbane, Australia*, ed. E. S. Delfosse, pp. 563–574. Melbourne, Australia: Commonwealth Scientific and Industrial Research Organization.
- Dennill, G. B. (1985). The effect of the gall wasp *Trichilogaster acaciaelongifoliae* (Hymenoptera: Pteromalidae) on reproductive potential and vegetative growth of the weed *Acacia longifolia*. *Agriculture, Ecosystems & Environment*, **14**, 53–61.
- Dietrick, E. J., Schlinger, E. I. & Garber, M. J. (1960). Sampling insect populations. *California Agriculture*, **Jan.**, 9–11.
- Dirsh, V. M. (1965). *The African Genera of Acridoidea*. Cambridge: Cambridge University Press.
- Dixon, A. F. G. (2000). *Insect Predator-Prey Dynamics: Ladybird Beetles and Biological Control*. Cambridge: Cambridge University Press.
- Dixon, B. (1994). Keeping an eye on *B. thuringiensis*. *BioTechnology*, **12**, 435.
- Dobson, A. P. (1988). Restoring island ecosystems: the potential of parasites to control introduced mammals. *Conservation Biology*, **2**, 31–39.

- Douglas, A. E. (1998). Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria *Buchnera*. *Annual Review of Entomology*, **43**, 17–37.
- Duetting, P. S. (2002). Effect of Field Pea Surface Wax Variation on Infection of the Pea Aphid by the Fungal Pathogen, *Pandora neoaphidis*. M.S. thesis, University of Idaho.
- Dyer, L. A. (1995). Tasty generalists and nasty specialists? Antipredator mechanisms in tropical lepidopteran larvae. *Ecology*, **76**, 1,483–1,496.
- Dyer, L. A. & Gentry, G. (1999). Predicting natural-enemy responses to herbivores in natural and managed systems. *Ecological Applications*, **9**, 402–408.
- Ehler, L. E. (1998). Conservation biological control: past, present, and future. In *Conservation Biological Control*, ed. P. Barbosa, pp. 1–8. San Diego, CA: Academic Press.
- Eigenbrode, S. D., Castagnola, T., Roux, M.-B. & Steljes, L. (1996). Mobility of three generalist predators is greater on cabbage with glossy leaf wax than on cabbage with a wax bloom. *Entomologia Experimentalis et Applicata*, **81**, 335–343.
- Eilenberg, J., Enkegaard, A., Vestergaard, S. & Jensen, B. (2000). Biocontrol of pests on plant crops in Denmark: present status and future potential. *Biocontrol Science and Technology*, **10**, 703–716.
- Eilenberg, J., Hajek, A. & Lomer, C. (2001). Suggestions for unifying the terminology in biological control. *BioControl*, **46**, 387–400.
- Elad, Y., Chet, I., Boyle, P. & Henis, Y. (1983). Parasitism of *Trichoderma* spp. on *Rhizoctonia solani* and *Sclerotium rolfsii* – scanning electron microscopy and fluorescence microscopy. *Phytopathology*, **73**, 85–88.
- Elkinton, J. S., Healy, W. M., Buonaccorsi, J. P., Boettner, G. H., Hazzard, A. M., Smith, H. R. & Liebhold, A. M. (1996). Interactions among gypsy moths, white-footed mice, and acorns. *Ecology*, **77**, 2,332–2,342.
- Embree, D. G. (1966). The role of introduced parasites in the control of the winter moth in Nova Scotia. *Canadian Entomologist*, **98**, 1,159–1,168.
- Entwistle, P. F. (1998). A world survey of virus control of insect pests. In *Insect Viruses and Pest Management*, ed. F. R. Hunter-Fujita, P. F. Entwistle, H. F. Evans & N. E. Crook, pp. 188–204. Chichester, UK: Wiley.
- Eriksson, J., Khortstam, K. & Ryvarden, L. (1981). *The Corticiaceae of North Europe*. Oslo, Norway: Fungiflora.
- Essig, E. O. (1942). *College Entomology*. New York: MacMillan.
- Federici, B. (1999). *Bacillus thuringiensis* in biological control. In *Handbook of Biological Control*, ed. T. S. Bellows & T. W. Fisher, pp. 575–593. San Diego, CA: Academic Press.
- Feitelson, J. S., Payne, J. & Kim, L. (1992). *Bacillus thuringiensis*: insects and beyond. *BioTechnology*, **10**, 271–275.
- Felske, A. & Akkermans, A. D. L. (1998). Spatial homogeneity of abundant bacterial 16S rRNA molecules in grassland soils. *Microbial Ecology*, **36**, 31–36.
- Fenner, F. & Fantini, B. (1999). *Biological Control of Vertebrate Pests: The History of Myxomatosis – An Experiment in Evolution*. Wallingford, UK: CABI Publishing.
- Fenner, F. & Myers, K. (1978). Myxoma virus and myxomatosis in retrospect: the first quarter century of a new disease. In *Viruses and Environment*, ed. E. Kurstak & K. Maramorosch, pp. 539–570. New York: Academic Press.

- Finch, S. & Collier, R. H. (2000). Host-plant selection by insects – a theory based on “appropriate/inappropriate landings” by pest insects of cruciferous plants. *Entomologia Experimentalis et Applicata*, **96**, 91–102.
- Flint, M. L. & Dreistadt, S. H. (1998). *Natural Enemies Handbook: The Illustrated Guide to Biological Control*. University of California Statewide IPM Project Publication 3386.
- Flint, M. L. & van den Bosch, R. (1981). *Introduction to Integrated Pest Management*. New York: Plenum Press.
- Follett, P. A., Duan, J., Messing, R. H. & Jones, V. P. (2000). Parasitoid drift after biological control introductions: re-examining Pandora’s box. *American Entomologist*, **46**, 82–94.
- Food and Agriculture Organization of the United Nations (FAO) (1997). Code of conduct for the import and release of exotic biological control agents. *Biocontrol News and Information*, **18**, 119N–124N.
- Fowler, S. V., Syrett, P. & Hill, R. L. (2000). Success and safety in the biological control of environmental weeds in New Zealand. *Austral Ecology*, **25**, 553–562.
- Friedman, M. J. (1990). Commercial production and development. In *Entomopathogenic Nematodes for Biological Control*, ed. R. Gaugler & H. K. Kaya, pp. 153–172. Boca Raton, FL: CRC Press.
- Fulbright, D. W. (1999). Hypovirulence to control fungal pathogenesis. In *Handbook of Biological Control*, ed. T. S. Bellows & T. W. Fisher, pp. 691–712. San Diego, CA: Academic Press.
- Funasaki, G. Y., Lai, P.-Y., Nakahara, L. M., Beardsley, J. W. & Ota, A. K. (1988). A review of biological control introductions in Hawaii: 1890 to 1985. *Proceedings of the Hawaiian Entomological Society*, **28**, 105–160.
- Fuxa, J. R. (1987). Ecological considerations for the use of entomopathogens in IPM. *Annual Review of Entomology*, **32**, 225–251.
- (1998). Environmental manipulation for microbial control of insects. In *Conservation Biological Control*, ed. P. Barbosa, pp. 255–268. San Diego, CA: Academic Press.
- Gage, S. H. & Haynes, D. L. (1975). Emergence under natural and manipulated conditions of *Tetrastichus julis*, an introduced larval parasite of the cereal leaf beetle, with reference to regional population management. *Environmental Entomology*, **4**, 425–434.
- Garcia, R. & Legner, E. F. (1999). Biological control of medical and veterinary pests. In *Handbook of Biological Control*, ed. T. S. Bellows & T. W. Fisher, pp. 935–953. San Diego, CA: Academic Press.
- Garcia, R., Caltagirone, L. E. & Gutierrez, A. P. (1988). Comments on a redefinition of biological control. *BioScience*, **38**, 692–694.
- Gause, G. F. (1934). *The Struggle for Existence*. Baltimore, MD: Williams and Wilkins.
- Gelernter, W. D. & Lomer, C. J. (2000). Success in biological control of above-ground insects by pathogens. In *Biological Control: Measures of Success*, ed. G. Gurr & W. Wratten, pp. 297–322. Dordrecht, NL: Kluwer Academic Publishers.
- Glare, T. R. & O’Callaghan, M. (2000). *Bacillus thuringiensis: Biology, Ecology and Safety*. Chichester, UK: Wiley & Sons.
- Goeden, R. D. & Andrés, L. A. (1999). Biological control of weeds in terrestrial and aquatic environments. In *Handbook of Biological Control*, ed. T. S. Bellows & T. W. Fisher, pp. 871–890. San Diego, CA: Academic Press.

- Goldberg, L. J. & Margalit, J. (1977). A bacterial spore demonstrating rapid larvicidal activity against *Anopheles sergentii*, *Uranotaenia unguiculata*, *Culex univittatus*, *Aedes aegypti*, and *Culex pipiens*. *Mosquito News*, **37**, 355–358.
- Gordh, G., Legner, E. F. & Caltagirone, L. E. (1999). Biology of parasitic Hymenoptera. In *Handbook of Biological Control*, ed. T. S. Bellows & T. W. Fisher, pp. 355–381. San Diego, CA: Academic Press.
- Graham, F. (1970). *Since Silent Spring*. Boston, MA: Houghton-Mifflin.
- Greathead, D. J. (1994). History of biological control. *Antenna*, **18**, 187–199.
- Greathead, D. J. & Greathead, A. H. (1992). Biological control of insect pests by insect parasitoids and predators: the BIOCAT database. *Biocontrol News & Information*, **13**, 61N–68N.
- Green, D. S. (1984). A proposed origin of the coffee leaf-miner, *Leucoptera coffeella* (Guérin-Méneville) (Lepidoptera: Lyonetiidae). *American Entomologist*, **30**(1), 30–31.
- Grewal, P. S. (2002). Formulation and application technology. In *Entomopathogenic Nematology*, ed. R. Gaugler, pp. 265–287. Wallingford, UK: CABI Publishing.
- Grime, J. P. (1977). Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist*, **111**, 1,169–1,194.
- Griswold, G. H. (1929). On the bionomics of a primary parasite and of two hyperparasites of the geranium aphid. *Annals of the Entomological Society of America*, **22**, 438–457.
- Gross, P. (1991). Influence of target pest feeding niche on success rates in classical biological control. *Environmental Entomology*, **20**, 1,217–1,227.
- Gullan, P. J. & Cranston, P. S. (2000). *The Insects: An Outline of Entomology*, 2nd edn. Oxford, UK: Blackwell Science.
- Gurr, G. M. & Wratten, S. D. (eds.) (2000). *Biological Control: Measures of Success*. Dordrecht, NL: Kluwer Academic Publishers.
- Gurr, G. M., Barlow, N., Memmott, J., Wratten, S. D. & Greathead, D. J. (2000a). A history of methodological, theoretical and empirical approaches to biological control. In *Biological Control: Measures of Success*, ed. G. M. Gurr & S. D. Wratten, pp. 3–37. Dordrecht, NL: Kluwer Academic Publishers.
- Gurr, G. M., Wratten, S. D. & Barbosa, P. (2000b). Success in conservation biological control of arthropods. In *Biological Control: Measures of Success*, ed. G. M. Gurr & S. D. Wratten, pp. 105–132. Dordrecht, NL: Kluwer Academic Publishers.
- Hagen, K. S., Sawall, Jr., E. F. & Tassen, R. L. (1970). The use of food sprays to increase effectiveness of entomophagous insects. *Proceedings of the Tall Timbers Conference on Ecological Animal Control by Habitat Management*, vol. 2, ed. R. Komarek, pp. 59–81. Tallahassee, FL: Tall Timbers Research Station.
- Hajek, A. E. (1999). Pathology and epizootiology of the lepidoptera-specific mycopathogen *Entomophaga maimaiga*. *Microbiology and Molecular Biology Reviews*, **63**, 814–835.
- Hajek, A. E., Wraight, S. P. & Vandenberg, J. D. (2001). Control of arthropods using pathogenic fungi. In *Bio-Exploitation of Fungi*, ed. S. B. Pointing & K. D. Hyde, pp. 309–347. Hong Kong: Fungal Diversity Press.
- Hajek, A. E., Delalibera Jr., I. & Butler, L. (2003). Entomopathogenic fungi as classical biological control agents. In *Environmental Impacts of Microbial Insecticides*, ed. H. M. T. Hokkanen & A. E. Hajek (2003). Dordrecht, NL: Kluwer Academic Publishers.

- Hall, F. R. & Menn, J. J. (1999). *Biopesticides, Use and Delivery*. Totowa, NJ: Humana Press.
- Handelsman, J. (2002). Future trends in biocontrol. In *Biological Control of Crop Diseases*, ed. S. S. Gnanamanickam, pp. 443–448. New York: Dekker.
- Harman, G. E. (2000). Myths and dogmas of biocontrol: Changes in perceptions derived from research on *Trichoderma harzianum* T-22. *Plant Disease*, **84**, 377–393.
- Harris, J. (2000). *Chemical Pesticide Markets, Health Risks, and Residues*. Wallingford, UK: CABI Publishing.
- Harris, P. (1981). Stress as a strategy in the biological control of weeds. In *Biological Control in Plant Production*, ed. G. C. Papavizas, pp. 333–340. Totowa, NJ: Allanheld.
- (1986). Biological control of weeds. In *Biological Plant and Health Protection, Biological Control of Plant Pests and of Vectors of Human and Animal Diseases*, ed. J. M. Franz, pp. 123–138. Stuttgart: G. Fischer.
- Harris, P., Wilkinson, A. T. S., Thompson, L. S. & Neary, M. (1978). Interaction between the cinnabar moth, *Tyria jacobaeae* L. (Lep., Arctiidae) and ragwort, *Senecio jacobaea* L. (Compositae) in Canada. *Proceedings of the Fourth International Symposium on Biological Control of Weeds*, ed. T. E. Freeman, p. 174–180. Gainesville, FL: Center for Environmental Programs, Institute of Food and Agricultural Sciences, University of Florida.
- Hartley, S. E. & Jones, C. G. (1997). Plant chemistry and herbivory, or why the world is green. In *Plant Ecology*, 2nd edn, ed. M. J. Crawley, pp. 284–324. Oxford: Blackwell.
- Hawkins, B. A. & Marino, P. C. (1997). The colonization of native phytophagous insects in North America by exotic parasitoids. *Oecologia*, **112**, 566–571.
- Hawkins, B. A., Mills, N. J., Jervis, M. A. & Price, P. W. (1999). Is the biological control of insects a natural phenomenon? *Oikos*, **86**, 493–506.
- Henry, J. E. & Oma, E. A. (1981). Pest control by *Nosema locustae*, a pathogen of grasshoppers and crickets. In *Microbial Control of Pests and Plant Diseases 1970–1980*, ed. H. D. Burges, pp. 573–586. New York: Academic Press.
- Herren, H. R., Bird, T. J. & Nadel, D. J. (1987). Technology for automated aerial release of natural enemies of the cassava mealybug and cassava green mite. *Insect Science and Application*, **8**, 883–885.
- Hickman, J. M. & Wratten, S. D. (1996). Use of *Phacelia tanacetifolia* strips to enhance biological control of aphids by hoverfly larvae in cereal fields. *Journal of Economic Entomology*, **89**, 832–840.
- Higley, L. G. & Pedigo, L. P. (ed.) (1996). *Economic Thresholds for Integrated Pest Management*. Lincoln, NE: University of Nebraska Press.
- Hoch, H. C. & Fuller, M. S. (1977). Mycoparasitic relationships. I. Morphological features of interactions between *Pythium acanthicum* and several fungal hosts. *Archives of Microbiology*, **111**, 207–224.
- Hochberg, M. E. (1989). The potential role of pathogens in biological control. *Nature*, **337**, 262–264.
- Hoddle, M. S. (1999). Biological control of vertebrate pests. In *Handbook of Biological Control: Principles and Applications of Biological Control*, ed. T. S. Bellows & T. W. Fisher, pp. 955–975. San Diego, CA: Academic Press.
- Hoffmann, J. H. (1995). Biological control of weeds: the way forward, a South African perspective. *BCPC Symposium Proceedings*, **64**, 77–89.
- Hoffmann, J. H. & Moran, V. C. (1992). Oviposition patterns and the supplementary role of a seed-feeding weevil, *Rhyssomatus marginatus*

- (Coleoptera: Curculionidae), in the biological control of a perennial leguminous weed, *Sesbania punicea*. *Bulletin of Entomological Research*, **82**, 343–347.
- (1995). Localized failure of a weed biological control agent attributed to insecticide drift. *Agriculture, Ecosystems & Environment*, **52**, 197–203.
- Hoffmann, M. P. & Frodsham, A. C. (1993). *Natural Enemies of Vegetable Insect Pests*. Ithaca, NY: Cornell Cooperative Extension.
- Hokkanen, H. M. T. & Lynch, J. M. (ed.) (1995). *Biological Control: Benefits and Risks*. Cambridge: Cambridge University Press.
- Hokkanen, H. & Pimentel, D. (1984). New approach for selecting biological control agents. *Canadian Entomologist*, **116**, 1,109–1,121.
- (1986). New associations in biological control: theory and practice. *Canadian Entomologist*, **121**, 829–840.
- Holling, C. S. 1966. The functional response of invertebrate predators to prey density. *Memoirs of the Canadian Entomological Society*, **48**, 3–86.
- Holloway, J. K. (1957). Weed control by insect. *Scientific American*, **197**, 56–62.
- Holm, L. G., Plucknett, D. L., Pancho, J. V. & Herberger, J. P. (1977). *The World's Worst Weeds. Distribution and Biology*. Honolulu, HI: University of Hawaii Press.
- Howarth, F. G. (1983). Biological control: panacea or Pandora's box? *Proceedings of the Hawaiian Entomological Society*, **24**, 239–244.
- (1991). Environmental impacts of classical biological control. *Annual Review of Entomology*, **36**, 485–510.
- Hoy, M. A. (1985). Recent advances in genetics and genetic improvement of the Phytoseiidae. *Annual Review of Entomology*, **30**, 345–370.
- (1993). Biological control in U.S. agriculture: back to the future. *American Entomologist*, **39**, 140–150.
- Huffaker, C. B. (1958). Experimental studies on predation: dispersion factors and predator-prey oscillations. *Hilgardia*, **27**, 343–383.
- Huffaker, C. B., Shea, K. P. & Herman, S. G. (1963). Experimental studies on predation: complex dispersion and levels of food in an acarine predator-prey interaction. *Hilgardia*, **34**, 305–330.
- Huffaker, C. B., Messenger, P. S. & DeBach, P. (1971). The natural enemy component in natural control and the theory of biological control. In *Biological Control*, ed. C. B. Huffaker, pp. 16–67. New York: Plenum Press.
- Hughes, C. E. (1995). Protocols for plant introductions with particular reference to forestry: changing perspectives on risks to biodiversity and economic development. *Proceedings Brighton Crop Protection Conference Symposium: Weeds in a Changing World*, vol. 64, pp. 15–32. Farnham, UK: British Crop Protection Council.
- Hull, L. A., Hickey, K. D. & Kanour, W. W. (1983). Pesticide usage patterns and associated pest damage in commercial apple orchards of Pennsylvania. *Journal of Economic Entomology*, **76**, 577–583.
- Hunter-Fujita, F. R., Entwistle, P. F., Evans, H. F. & Crook, N. E. (1998). *Insect Viruses and Pest Management*. Chichester, UK: Wiley.
- Hutchinson, G. E. (1957). *A Treatise on Limnology*. Vol. 2. *Introduction to Lake Biology and the Limnoplankton*. New York: John Wiley.
- Jackson, T. A., Pearson, J. F., O'Callaghan, M. O., Mahanty, H. K. & Willocks, M. J. (1992). Pathogen to product – development of *Serratia entomophila* (Enterobacteriaceae) as a commercial biological control agent for the New Zealand grass grub (*Costelytra zealandica*). In *Use of Pathogens in Scarab Pest Management*, ed. T. R. Glare & T. A. Jackson, pp. 191–198. Andover, UK: Intercept.

- Jordan, D. S. & Evermann, B. W. (1900). The fishes of North and Middle America. *Bulletin of the United States National Museum*, **47**, Part IV: plate CXIII.
- Julien, M. H. (ed.) (1992). *Biological Control of Weeds: A World Catalogue of Agents and Their Target Weeds*, 3rd edn. Wallingford, UK: CABI Publishing.
- Julien, M. & Griffiths, M. W. (ed.) (1998). *Biological Control of Weeds: A World Catalogue of Agents and Their Target Weeds*, 4th edn. Wallingford, UK: CABI Publishing.
- Julien, M. & White, G. (1997). *Biological Control of Weeds: Theory and Practical Application*. ACIAR Monograph 49. Canberra, Australia: Australian Centre for International Agricultural Research.
- Kerr, A. (1980). Biological control of crown gall through production of agrocin 84. *Plant Disease*, **64**, 25–30.
- Kerry, B. R. (2000). Rhizosphere interactions and the exploitation of microbial agents for the biological control of plant-parasitic nematodes. *Annual Review of Phytopathology*, **38**, 423–441.
- (2001). Exploitation of the nematophagous fungal *Verticillium chlamydosporium* Goddard for the biological control of root-knot nematodes (*Meloidogyne* spp.). In *Fungi as Biocontrol Agents: Progress, Problems and Potential*, ed. T. M. Butt, C. Jackson & N. Magan, pp. 155–167. Wallingford, UK: CAB International.
- Kessler, A. & Baldwin, I. T. (2001). Defensive function of herbivore-induced plant volatile emission in nature. *Science*, **291**, 2,141–2,144.
- Kiritani, K., Kawahara, S., Sasaba, T. & Nakasuji, F. (1972). Quantitative evaluation of predation by spiders on the green rice leafhopper, *Nephotettix cincticeps* Uhler, a sight count method. *Researches on Population Ecology*, **13**, 187–200.
- Knull, J. N. (1951). The checkered beetles of Ohio. *Ohio Biological Survey Bulletin*, **42**, 267–350.
- Kogan, M. (1994). Plant resistance in pest management. In *Introduction to Insect Pest Management*, 3rd edn, ed. R. L. Metcalf & W. H. Luckmann, pp. 73–128. New York: John Wiley & Sons.
- (1998). Integrated pest management: historical perspectives and contemporary developments. *Annual Review of Entomology*, **43**, 243–270.
- Kok, L. T. (2001). Classical biological control of nodding and plumeless thistles. *Biological Control*, **21**, 206–213.
- Krebs, C. J. (2001). *Ecology: The Experimental Analysis of Distribution and Abundance*, 5th edn. San Francisco, CA: Benjamin Cummings.
- Krieg, A., Huger, A. M., Langenbruch, G. A. & Schnetter, W. (1983). *Bacillus thuringiensis* var. *tenebrionis*: a new pathotype effective against larvae of Coleoptera. *Zeitschrift für Angewandte Entomologie*, **96**, 500–508.
- Lafferty, K. D. & Kuris, A. M. (1996). Biological control of marine pests. *Ecology*, **77**, 1,989–2,000.
- Landis, D. A., Wratten, S. D. & Gurr, G. M. (2000). Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology*, **45**, 175–201.
- Lawton, J. H. (1990). Biological control of plants: a review of generalisations, rules and principles using insects as agents. *New Zealand Forest Service, Forest Research Institute Bulletin*, **155**, 3–17.
- Lawton, J. H. & McNeill, S. (1979). Between the devil and the deep blue sea: on the problem of being an herbivore. In *Population Dynamics*, ed. K. Anderson, B. Turner & L. R. Taylor, pp. 223–245. Oxford: Blackwell.

- Leake, D. V., Leake, J. B. & Roeder, M. L. (1993). *Desert and Mountain Plants of the Southwest*. Norman, OK: Oklahoma University Press.
- Lewis, S. (1989). *Cane Toads: An Unnatural History*. New York: Dolphin/Doubleday.
- Lewis, W. J., van Lenteren, J. C., Phatak, S. C. & Tumlinson, III, J. H. (1997). A total system approach to sustainable pest management. *Proceedings of the National Academy of Sciences, USA*, **94**, 12,243–12,248.
- Li, L.-Y. (1994). Worldwide use of *Trichogramma* for biological control of different crops: a survey. In *Biological Control with Egg Parasitoids*, ed. E. Wajnberg & S. A. Hassan, pp. 37–53. Wallingford, UK: CAB International.
- Liang, W. & Huang, M. (1994). Influence of citrus orchard ground cover plants on arthropod communities in China: a review. *Agriculture, Ecosystems & Environment*, **45**, 175–201.
- Lisansky, S. (1997). Microbial biopesticides. In *Microbial Insecticides: Novelty or necessity?* British Crop Protection Council Symposium Proceedings, **68**, 3–10.
- Lomer, C. J., Thomas, M. B., Douro-Kpindou, O.-K., Gbongboui, C., Godonou, I., Langewald, J. & Shah, P. A. (1997). Control of grasshoppers, particularly *Hieroglyphus daganensis*, in northern Benin using *Metarhizium anisopliae*. *Memoirs of the Entomological Society of Canada*, **171**, 301–311.
- Lomer, C. J., Bateman, R. P., Johnson, D. L., Langewald, J. & Thomas, M. (2001). Biological control of locusts and grasshoppers. *Annual Review of Entomology*, **46**, 667–702.
- Loosey, J. E. & Denno, R. F. (1998). Positive predator–predator interactions: enhanced predation rates and synergistic suppression of aphid populations. *Ecology*, **79**, 2,143–2,152.
- Louda, S. M., Kendall, D., Connor, J. & Simberloff, D. (1997). Ecological effects of an insect introduced for the biological control of weeds. *Science*, **277**, 1,088–1,090.
- Louda, S. M., Pemberton, R. W., Johnson, M. T. & Follett, P. A. (2003). Non-target effects – The Achilles’ heel of biological control? *Annual Review of Entomology*, **48**, 365–396.
- Luck, R. F., Shepard, B. M. & Kenmore, P. E. (1988). Experimental methods for evaluating arthropod natural enemies. *Annual Review of Entomology*, **33**, 367–391.
- Luckmann, W. H. & Metcalf, R. L. (1994). The pest management concept. In *Introduction to Insect Pest Management* 3rd edn, ed. R. L. Metcalf & W. H. Luckmann, pp. 1–34. New York: Wiley.
- Lumsden, R. D. & Vaughn, J. L. (ed.) (1993). *Pest Management: Biologically Based Technologies*. Washington, DC: American Chemical Society.
- Lym, R. G. & Zollinger, R. K. (1995). *Integrated Management of Leafy Spurge*. North Dakota State Extension Service Publication W-866, 8 pp. Fargo, ND: North Dakota State University Extension Service.
- Lynch, L. D. & Thomas, M. B. (2000). Nontarget effects in the biocontrol of insects with insects, nematodes and microbial agents: the evidence. *Biocontrol News & Information*, **21**, 117N–130N.
- Malcolm, S. B. (1992). Prey defence and predator foraging. In *Natural Enemies: The Population Biology of Predators, Parasites and Diseases*, ed. M. J. Crawley, pp. 458–475. Oxford, UK: Blackwell Scientific Publications.
- Malecki, R. A., Blossey, B., Hight, S. D., Schroeder, D., Kok, L. T. & Coulson, J. R. (1993). Biological control of purple loosestrife. *BioScience*, **43**, 680–686.

- Marshall, J. D. & Fenner, F. (1960). Studies in the epidemiology of infectious myxomatosis of rabbits. VII. The virulence of strains of myxoma virus recovered from Australian wild rabbits between 1951 and 1959. *Journal of Hygiene*, **58**, 485–488.
- Mathre, D. E., Cook, R. J. & Callan, N. W. (1999). From discovery to use, traversing the world of commercializing biocontrol agents for plant disease control. *Plant Disease*, **83**, 972–983.
- Matteson, P. C. (1995). The “50% pesticide cuts” in Europe: a glimpse of our future? *American Entomologist*, **41**, 210–220.
- Mattson, W. J. (1980). Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics*, **11**, 119–161.
- Mayhew, P. J. & Blackburn, T. M. (1999). Does development mode organize life-history traits in the parasitoid Hymenoptera? *Journal of Animal Ecology*, **68**, 906–916.
- McCoy, C., Shapiro, D. I. & Duncan, L. W. (2000). Application and evaluation of entomopathogens for citrus pest control. In *Field Manual of Techniques in Invertebrate Pathology*, ed. L. A. Lacey & H. K. Kaya, pp. 577–595. Dordrecht, NL: Kluwer Academic Publishers.
- McEvoy, P. B. & Coombs, E. M. (1999). Biological control of plant invaders: regional patterns, field experiments, and structured population models. *Ecological Applications*, **9**, 387–401.
- McEvoy, P. B., Rudd, N. T., Cox, C. S. & Huso, M. (1993). Disturbance, competition, and herbivory effects on ragwort *Senecio jacobaea* populations. *Ecological Monographs*, **63**, 55–75.
- McFadyen, R. E. C. (1998). Biological control of weeds. *Annual Review of Entomology*, **43**, 369–393.
- Milner, R. J. (1997). *Metarhizium flavoviride* (F1985) as a promising mycoinsecticide for Australian acridids. *Memoirs of the Entomological Society of Canada*, **171**, 287–300.
- Morrison, L. W., Dall’aglio-Holvercem, C. G. & Gilbert, L. E. (1997). Oviposition behavior and development of *Pseudacteon* flies (Diptera, Phoridae), parasitoids of *Solenopsis* fire ants (Hymenoptera, Formicidae). *Environmental Entomology*, **26**, 716–724.
- Moscardi, F. (1999). Assessment of the application of baculoviruses for control of Lepidoptera. *Annual Review of Entomology*, **44**, 257–289.
- Mota-Sanchez, D., Bills, P. S. & Whalon, M. E. (2002). Arthropod resistance to pesticides: status and overview. In *Pesticides in Agriculture and the Environment*, ed. W. B. Wheeler, pp. 241–272. New York: Dekker.
- Murdoch, W. W. & Briggs, C. J. (1996). Theory for biological control: recent developments. *Ecology*, **77**, 2,001–2,013.
- Murdoch, W. W., Chesson, J. & Chesson, P. L. (1985). Biological control theory and practice. *American Naturalist*, **125**, 344–366.
- Murdoch, W. W., Luck, R. F., Swarbrick, S. L., Walde, S., Yu, D. S. & Reeve, J. D. (1995). Regulation of an insect population under biological control. *Ecology*, **76**, 206–217.
- Murphy, B. C., Rosenheim, J. A., Granett, J., Pickett, C. H. & Dowell, R. V. (1998). Measuring the impact of a natural enemy refuge: the prune tree/vineyard example. In *Enhancing Biological Control: Habitat Management to Promote Natural Enemies of Agricultural Pests*, ed. C. H. Pickett & R. L. Bugg, pp. 297–309. Berkeley, CA: University of California Press.
- Murphy, G. D., Ferguson, G., Frey, K., Lambert, L., Mann, M. & Matteoni, J. (2002). The use of biological control in Canadian greenhouse crops. *IOBC WPRS Bulletin*, **25(1)**, 193–196.

- Murray, E. (1993). The sinister snail. *Endeavour (Cambridge)*, **17**, 78–83.
- Myers, J. H. & Harris, P. (1980). Distribution of *Urophora* galls in flowers heads of diffuse and spotted knapweed in British Columbia. *Journal of Applied Ecology*, **17**, 359–367.
- Myers, J. H. & Risley, C. (2000). Why reduced seed production is not necessarily translated into successful biological weed control. In *Proceedings, X International Symposium on Biological Control of Weeds*. Bozeman, Montana, ed. N. R. Spencer, pp. 569–581. Bozeman, MT: Montana State University.
- National Academy of Sciences (US) (1988). *Research Briefings 1987: Report of the Research Briefing Panel on Biological Control in Managed Ecosystems*. Washington, DC: National Academy Press.
- National Research Council (US), Committee on Pest and Pathogen Control Through Management of Biological Control Agents and Enhanced Cycles and Natural Processes. (1996). *Ecologically Based Pest Management: New Solutions for a New Century*. Washington, DC: National Academy Press.
- Neilson, M. M. & Elgee, D. E. (1965). An unusual increase in spruce sawfly numbers in New Brunswick. *Bi-Monthly Progress Report, Canada Department of Forestry*, **21**(2), 1.
- Neuenschwander, P. & Herren, H. (1988). Biological control of the cassava mealybug, *Phenacoccus manihoti*, by the exotic parasitoid *Epidinocarsis lopezi* in Africa. *Philosophical Transactions of the Royal Society of London, B*, **318**, 319–333.
- Newhouse, J. R. (1990). Chestnut blight. *Scientific American*, **263**, 106–111.
- Newman, R. M., Thompson, D. C. & Richman, D. B. (1998). Conservation strategies for the biological control of weeds. In *Conservation Biological Control*, ed. P. Barbosa, pp. 371–396. San Diego, CA: Academic Press.
- Newsome, A. (1990). The control of vertebrate pests by vertebrate predators. *Trends in Ecology and Evolution*, **5**, 187–191.
- Nicholson, A. J. & Bailey, V. A. (1935). The balance of animal populations. Part I. *Proceedings of the Zoological Society of London*. **Part 3**, 551–598.
- Nilsson, C. (1985). Impact of plough in on emergence of pollen beetle parasitoids after hibernation. *Zeitschrift für Angewandte Entomologie*, **100**, 302–308.
- Nordlund, D. A. (1996). Biological control, integrated pest management and conceptual models. *Biocontrol News & Information*, **17**, 35N–44N.
- Nyrop, J., English-Loeb, G. & Roda, A. (1998). Conservation biological control of spider mites in perennial cropping systems. In *Conservation Biological Control*, ed. P. Barbosa, pp. 307–333. San Diego, CA: Academic Press.
- Office of Technology Assessment (OTA; US Congress, 1995). *Biologically Based Technologies for Pest Control*. OTA-ENV-636. Washington, DC: US Government Printing Office.
- Parer, I., Conolly, D. & Sobey, W. R. (1985). Myxomatosis: the effects of annual introductions of an immunizing strain and a highly virulent strain of myxoma virus into rabbit populations in Urana, N.S.W. *Australian Wildlife Research*, **12**, 407–423.
- Parrella, M. P., Hansen, L. S. & van Lenteren, J. (1999). Glasshouse environments. In *Handbook of Biological Control*, ed. T. S. Bellows & T. W. Fisher, pp. 819–839. San Diego, CA: Academic Press.
- Paulitz, T. C. & Bélanger, R. R. (2001). Biological control in greenhouse systems. *Annual Review of Phytopathology*, **39**, 103–133.
- Pedigo, L. P. (1996). *Entomology and Pest Management*, 2nd edn. Upper Saddle River, NJ: Prentice-Hall.

- Pemberton, R. W. (2000). Predictable risk to native plants in weed biological control. *Oecologia*, **125**, 489–494.
- Perfecto, I. & Castiñeiras, A. (1998). Deployment of the predaceous ants and their conservation in agroecosystems. In *Conservation Biological Control*, ed. P. Barbosa, pp. 269–289. San Diego, CA: Academic Press.
- Perkins, J. H. & Garcia, R. (1999). Social and economic factors affecting research and implementation of biological control. In *Handbook of Biological Control*, ed. T. S. Bellows & T. W. Fisher, pp. 993–1,009. San Diego, CA: Academic Press.
- Pickett, C. H. & Bugg, R. L. (ed.) (1998). *Enhancing Biological Control: Habitat Management to Promote Natural Enemies of Agricultural Pests*. Berkeley, CA: University of California Press.
- Pimentel, D. (ed.) (2002). *Biological Invasions: Economic and Environmental Costs of Alien Plant, Animal, and Microbe Species*. Boca Raton, FL: CRC Press.
- Pimentel, D., Acquay, H., Biltonen, M., Rice, P., Silva, M., Nelson, J., Lipner, V., Giordano, S., Horowitz, A. & D'Amore, M. (1992). Assessment of environmental and economic impacts of pesticide use. In *The Pesticide Question: Environment, Economics, and Ethics*, ed. D. Pimentel & H. Lehman, pp. 47–84. New York: Chapman & Hall.
- Poinar, G. O. (1979). *Nematodes for Biological Control of Insects*. Boca Raton, FL: CRC Press.
- Price, P. W. (1973). Parasitoid strategies and community organization. *Environmental Entomology*, **2**, 623–626.
- (1984). The concept of the ecosystem. In *Ecological Entomology*, ed. C. B. Huffaker & R. L. Rabb, pp. 19–50. New York: John Wiley & Sons.
- Quarles, W. (ed.) (2002). 2003 directory of least-toxic pest control products. *The IPM Practitioner*, **24** (11/12), 1–52.
- Rabb, R. L., Stinner, R. E. & van den Bosch, R. (1976). Conservation and augmentation of natural enemies. In *Theory and Practice of Biological Control*, ed. C. B. Huffaker & P. S. Messenger, pp. 233–254. New York: Academic Press.
- Reeve, J. D. & Murdoch, W. W. (1985). Aggregation by parasitoids in the successful control of the California red scale: A test of theory. *Journal of Animal Ecology*, **54**, 797–816.
- (1986). Biological control by the parasitoid *Aphytis melinus*, and population stability of the California red scale. *Journal of Animal Ecology*, **55**, 1,069–1,082.
- Rishbeth, J. R. (1975). Stump inoculation: a biological control of *Fomes annosus*. In *Biology and Control of Soil-borne Pathogens*, ed. G. W. Bruehl, pp. 158–162. St. Paul, MN: American Phytopathological Society.
- Ristaino, J. B. & Thomas, W. (1997). Agriculture, methyl bromide, and the ozone hole. Can we fill the gaps? *Plant Disease*, **81**, 964–977.
- Root, R. B. (1973). Organization of a plant–arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecological Monographs*, **43**, 95–124.
- Rosenheim, J. A. (1998). Higher-order predators and the regulation of insect herbivore populations. *Annual Review of Entomology*, **43**, 421–447.
- Roskopf, E. N., Charudattan, R. & Kadir, J. B. (1999). Use of plant pathogens in weed control. In *Handbook of Biological Control*, ed. T. S. Bellows & T. W. Fisher, pp. 891–918. San Diego, CA: Academic Press.
- Ruberson, J. R., Kring, T. J. & Elkassabany, N. (1998). Overwintering and the diapause syndrome of predatory Heteroptera. In *Predatory Heteroptera in*

- Agroecosystems: Their Biology and Use in Biological Control*, ed. M. Coll & J. R. Ruberson, pp. 46–69. Lanham, MD: Entomological Society of America.
- Rutz, D. A. & Watson, D. W. (1998). Parasitoids as a component in an integrated fly-management program on dairy farms. In *Mass-Reared Natural Enemies: Application, Regulation, and Needs*, ed. R. L. Ridgway, M. P. Hoffmann, M. N. Inscoe & C. S. Glenister, pp. 185–201. Lanham, MD: Entomological Society of America.
- Sasser, J. N. & Freckman, D. W. (1987). A world perspective on nematology: the role of the society. In *Vistas on Nematology: A Commemoration of the Twenty-fifth Anniversary of the Society of Nematologists*, ed. J. A. Veech & D. W. Dickson, pp. 7–14. Hyattsville, MD: Society of Nematologists.
- Schaab, R. (1996). *Economy and Ecology of Biological Control Activities in Africa. A Case Study on the Cassava Mealybug, Phenacoccus manihoti Mat. Ferr.* Germany: University of Hohenheim. 141 pp.
- Schlenger, E. I. & Dietrick, E. J. (1960). Biological control of insect pests aided by strip-farming alfalfa in experimental program. *California Agriculture*, Jan., 8–9 and 15.
- Schmidt, J. M. (1992). Host recognition and acceptance by *Trichogramma*. In *Biological Control with Egg Parasitoids*, ed. E. Wajnberg & S. A. Hassan, pp. 165–200. Wallingford, UK: CAB International.
- Schroeder, D. (1983). Biological control of weeds. In *Recent Advances in Weed Control*, ed. W. E. Fletcher, pp. 41–78. Farnham Royal, Slough, UK: Commonwealth Agricultural Bureau.
- Shaw, S. R. (1993). Observations on the ovipositional behaviour of *Neoneurus mantis*, an ant-associated parasitoid from Wyoming (Hymenoptera: Braconidae). *Journal of Insect Behavior*, **6**, 649–658.
- Shu, S., Swedenborg, P. D. & Jones, R. L. (1990). A kairomone for *Trichogramma nubilale* (Hymenoptera: Trichogrammatidae): isolation, identification, and synthesis. *Journal of Chemical Ecology*, **16**, 521–529.
- Shuler, M. L., Hammer, D. A., Granados, R. R. & Wood, H. A. (1995). Overview of baculovirus–insect culture system. In *Baculovirus Expression Systems and Biopesticides*, ed. M. L. Shuler, H. A. Wood, R. R. Granados & D. A. Hammer, pp. 1–11. New York: John Wiley & Sons.
- Silvestri, F. (1906). Contribuzioni alla conoscenza biologica degli imenotteri parassiti. I. Biologica del *Litomastix truncatellus* (Dalm.). *Bollettino del Laboratorio di Zoologia Generale e Agraria della Facolta Agraria in Portici*, **1**, 17–64.
- Slobodkin, L. B. (1988). Intellectual problems of applied ecology. *BioScience*, **38**, 337–342.
- Smart, G. C., Jr. (1995). Entomopathogenic nematodes for the biological control of insects. *Journal of Nematology*, **27**, 529–534.
- Smith, H. S. (1919). On some phases of insect control by the biological method. *Journal of Economic Entomology*, **12**, 288–292.
- Southwood, T. R. E. (1978). *Ecological Methods: With Practical Reference to the Study of Insect Populations*, 2nd edn. London: Chapman and Hall.
- Sprenkel, R. K., Brooks, W. M., Van Duyn, J. W. & Deitz, L. L. (1979). The effects of three cultural variables on the incidence of *Nomuraea rileyi*, a phytophagous Lepidoptera, and their predators on soybeans. *Environmental Entomology*, **3**, 334–339.
- Steinhaus, E. A. (1975). *Disease in a Minor Chord*. Columbus, OH: Ohio University Press.

- Stern, V. M., Smith, R. F., van den Bosch, R. & Hagen, K. S. (1959). The integration of chemical and biological control of the spotted alfalfa aphid. I. The integrated control concept. *Hilgardia*, **29**, 81–101.
- Stiling, P. (1990). Calculating the establishment rates of parasitoids in classical biological control. *American Entomologist*, **36**, 225–230.
- (1993). Why do natural enemies fail in classical biological control programs? *American Entomologist*, **39**, 31–37.
- Strand, M. R. & Pech, L. L. (1995). Immunological basis for compatibility in parasitoid–host relationships. *Annual Review of Entomology*, **40**, 31–56.
- Strong, D. R., Lawton, J. H. & Southwood, T. R. E. (1984). *Insects on Plants*. Oxford: Oxford University Press.
- Sutton, J. C. & Peng, G. (1993). Manipulation and vectoring of biocontrol organisms to manage foliage and fruit diseases in cropping systems. *Annual Review of Phytopathology*, **31**, 473–493.
- Swezey, O. H. (1943). Biographical sketch of the work of Albert Koebele in Hawaii. *Hawaiian Planters' Record*, **47**, 5–8.
- Symondson, W. O. C., Sunderland, K. D. & Greenstone, M. H. (2002). Can generalist predators be effective biocontrol agents? *Annual Review of Entomology*, **47**, 561–594.
- Tanada, Y. & Kaya, H. K. (1993). *Insect Pathology*. San Diego, CA: Academic Press.
- Tang, W. H. (1994). Yield-increasing bacteria (YIB) and biocontrol of sheath blight of rice. In *Improving Plant Productivity and Rhizosphere Bacteria*, ed. M. H. Ryder, P. M. Stephens & G. D. Bowen, pp. 267–273. Adelaide, Australia: CSIRO, Division of Soils.
- Tauber, M. J., Tauber, C. A., Daane, K. M. & Hagen, K. S. (2000). Commercialization of predators: recent lessons from green lacewings (Neuroptera: Chrysopidae: *Chrysoperla*). *American Entomologist*, **46**, 26–38.
- TeBeest, D. O., Yang, X. B. & Cisar, C. R. (1992). The status of biological control of weeds with fungal pathogens. *Annual Review of Entomology*, **30**, 637–657.
- Thies, C. & Tscharntke, T. (1999). Landscape structure and biological control in agroecosystems. *Science*, **285**, 893–895.
- Thomas, M. B. (1999). Ecological approaches and the development of “truly integrated” pest management. *Proceedings of the National Academy of Sciences, USA*, **96**, 5,944–5,951.
- Thomas, M. B. & Willis, A. J. (1998). Biocontrol – risky but necessary? *Trends in Ecology and Evolution*, **13**, 325–329.
- Thomas, M. B., Wratten, S. D. & Sotherton, N. W. (1991). Creation of “island” habitats in farmland to manipulate populations of beneficial arthropods: predator densities and emigration. *Journal of Applied Ecology*, **28**, 906–917.
- Thomas, M. B., Wratten, S. D. & Sotherton, N. W. (1992). Creation of “island” habitats in farmland to manipulate populations of beneficial arthropods: predator densities and species composition. *Journal of Applied Ecology*, **29**, 524–531.
- Thomas, P. A. & Room, P. M. (1986). Taxonomy and control of *Salvinia molesta*. *Nature*, **320**, 581–584.
- Tisdell, C. (1990). Economic impact of biological control of weeds and insects. In *Critical Issues in Biological Control*, ed. M. Mackauer, L. E. Ehler & J. Roland, pp. 301–316. Andover, UK: Intercept.

- Topham, M. & Beardsley, J. W. (1975). Influence of nectar source plants on the New Guinea sugarcane weevil parasite, *Lixophaga sphenophori* (Villeneuve). *Proceedings of the Hawaiian Entomological Society*, **22**, 145–154.
- Tothill, J. D., Taylor, T. H. C. & Paine, R. W. (1930). *The Coconut Moth in Fiji. A History of its Control by Means of Parasites*. London: Imperial Bureau of Entomology.
- Traveset, A. (1990). Bruchid egg mortality on *Acacia farnesiana* caused by ants and abiotic factors. *Ecological Entomology*, **15**, 463–467.
- Trouvelot, B. (1931). Recherches sur les parasites et predateurs attaquant le doryphore en Amerique du Nord. *Annales des Épiphyties*, **17**, 408–445.
- Tumlinson, J. H., Lewis, W. J. & Vet, L. E. M. (1993). How parasitic wasps find their hosts. *Scientific American*, **268**, 100–106.
- Turchin, P., Taylor, A. E. & Reeve, J. D. (1999). Dynamical role of predators in population cycles of a forest insect: an experimental test. *Science*, **285**, 1,068–1,071.
- van Alphen, J. J. M. & Jervis, M. A. (1996). Foraging behaviour. In *Insect Natural Enemies: Practical Approaches to their Study and Evaluation*, ed. M. Jervis & N. Kidd, pp. 1–62. London: Chapman & Hall.
- van de Peer, Y., Ben-Ali, A. & Meyer, A. (2000). Microsporidia: accumulating molecular evidence that a group of amitochondriate and suspectedly primitive eukaryotes are just curious fungi. *Gene*, **246**, 1–8.
- van den Bosch, R. (1978). *The Pesticide Conspiracy*. New York: Doubleday.
- van den Bosch, R. & Hagen, K. S. (1966). Predaceous and parasitic arthropods in California cotton fields. *California Agricultural Experiment Station Bulletin* **8620**, 32 pp.
- van den Bosch, R. & Stern, V. (1969). The effect of harvesting practices on insect populations in alfalfa. *Proceedings of the Tall Timbers Conference on Ecological Animal Control*, **1**, 47–54.
- van den Bosch, R. & Telford, A. D. (1964). Environmental modification and biological control. In *Biological Control of Insect Pests and Weeds*, ed. P. H. DeBach, pp. 459–488. New York: Reinhold Publishing Corporation.
- van den Bosch, R., Messenger, P. S. & Gutierrez, A. P. (1982). *An Introduction to Biological Control*. New York: Plenum Press.
- Van Driesche, R. G. & Bellows, T. S., Jr. (1996). *Biological Control*. New York: Chapman & Hall.
- van Lenteren, J. C. (1980). Evaluation of control capabilities of natural enemies: does art have to become science? *Netherlands Journal of Zoology*, **30**, 369–381.
- (1997). Benefits and risks of introducing exotic macro-biological control agents into Europe. *Bulletin OEPP/EPPO*, **27**, 15–27.
- (2000). Success in biological control of arthropods by augmentation of natural enemies. In *Biological Control: Measures of Success*, ed. G. Gurr & S. Wratten, pp. 77–103. Dordrecht, NL: Kluwer Academic Publishers.
- van Lenteren, J. C. & Manzaroli, G. (1999). Evaluation and use of predators and parasitoids for biological control of pests in greenhouses. In *Integrated Pest and Disease Management in Greenhouse Crops*, ed. R. Albajes, M. Lodovica Gullino, J. C. van Lenteren & Y. Elad, pp. 183–201. Dordrecht, NL: Kluwer Academic Publishers.
- van Lenteren, J. C. & Martin, N. A. (1999). Biological control of whiteflies. In *Integrated Pest and Disease Management in Greenhouse Crops*, ed. R. Albajes,

- M. Lodovica Gullino, J. C. van Lenteren & Y. Elad, pp. 202–216. Dordrecht, NL: Kluwer Academic Publishers.
- van Lenteren, J. C., Roskam, M. M. & Timmer, R. (1997). Commercial mass production and pricing of organisms for biological control of pests in Europe. *Biological Control*, **10**, 143–149.
- van Lenteren, J. C., Hale, A., Klapwijk, J. N., van Schelt, J. & Steinberg, S. (2003). Guidelines for quality control of commercially produced natural enemies. In *Quality Control and Production of Biological Control Agents: Theory and Testing Procedures*, ed. J. C. van Lenteren, pp. 265–303. Wallingford, UK: CABI Publishing.
- Viggiani, G. (1964). La specializzazione entomoparassitica in alcuni Eulofidi (Hym., Chalcidoidea). *Entomophaga*, **9**, 111–118.
- Vittum, P. J., Villani, M. G. & Tashiro, H. (1999). *Turfgrass Insects of the United States and Canada*, 2nd edn. Ithaca, NY: Cornell University Press.
- Waage, J. (1990). Ecological theory and the selection of biological control agents. In *Critical Issues in Biological Control*, ed. M. Mackauer, L. E. Ehler & J. Roland, pp. 135–157. Andover, UK: Intercept.
- (1995). The use of exotic organisms as biopesticides: some issues. In *Biological Control: Benefits and Risks*, ed. H. M. T. Hokkanen & J. M. Lynch, pp. 93–100. Cambridge: Cambridge University Press.
- Wagner, D. L., Peacock, J. W., Carter, J. L. & Talley, S. E. (1996). Field assessment of *Bacillus thuringiensis* on nontarget Lepidoptera. *Environmental Entomology*, **25**, 1,444–1,454.
- Wapshere, A. J. (1989). A testing sequence for reducing rejection of potential biological control agents for weeds. *Annals of Applied Biology*, **114**, 515–526.
- Webster, F. M. (1909). Investigations of *Toxoptera graminum* and its parasites. *Annals of the Entomological Society of America*, **2**, 67–87.
- Weller, D. M., Raaijmakers, J. M., McSpadden Gardener, B. B. & Thomashow, L. S. (2002). Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annual Review of Phytopathology*, **40**, 309–348.
- Wetzstein, H. Y. & Phatak, S. C. (1987). Scanning electron microscopy of the uredinial stage of *Puccinia canaliculata* on yellow nutsedge, *Cyperus esculentus* (Cyperaceae). *American Journal of Botany*, **74**, 100–106.
- Whipps, J. M. & Davies, K. G. (2000). Success in biological control of plant pathogens and nematodes by microorganisms. In *Biological Control: Measures of Success*, ed. G. Gurr & S. Wratten, pp. 231–269. Dordrecht, NL: Kluwer Academic Publishers.
- White, G. (1997). Population ecology and biological control of weeds. In *Biological Control of Weeds: Theory and Practical Application*, eds. M. Julien & G. White, pp. 39–45. ACIAR Monograph No. 49.
- Wijnands, F. G. & Kroonen-Backbier, B. M. A. (1993). Management of farming systems to reduce pesticide inputs: the integrated approach. In *Modern Crop Protection: Developments and Perspectives*, ed. J. C. Zadoks, pp. 227–234. Wageningen, NL: Wageningen Pers.
- Woodburn, A. T. (1995). The market for agrochemicals present and future. *Proceedings of the Brighton Crop Protection Conference – Weeds 1*, pp. 121–128. Farnham, UK: British Crop Protection Council.
- World Health Organization (WHO). (1992). *WHO. Our Planet, Our Health*. Geneva: Report of the WHO Commission on Health and Environment.

- Wright, M. G., Kuhar, T. P., Hoffmann, M. P. & Chenus, S. A. (2002). Effect of inoculative releases of *Trichogramma ostrinae* on populations of *Ostrinia nubilalis* and damage to sweet corn and field corn. *Biological Control*, **23**, 149–155.
- Yaninek, J. S. & Hanna, R. (2003). Cassava green mite in Africa – a unique example of successful classical biological control of a mite pest on a continental scale. In *Biological Control in IPM Systems in Africa*, ed. P. Neuenschwander, C. Borgemeister & J. Langewald, Wallingford, UK: CAB International.
- Zelazny, B., Lolong, A. & Pattang, B. (1992). *Oryctes rhinoceros* (Coleoptera, Scarabaeidae) populations suppressed by a baculovirus. *Journal of Invertebrate Pathology*, **59**, 61–68.

Index

- abiotic factors 93–94
Acacia spp. 253
Acacia farnesiana 22
Acacia longifolia 243
Acacia saligna 256
acclimatization societies 16–17
Achatina fulica (giant African snail) 305
Achilles heel 22, 54, 228
acorns 122–123
Acridotheres tristis (mynah birds) 124
activation–inhibition hypothesis 226
Acyrtosiphon malvae 154, 156
Acyrtosiphon pisum (pea aphid) 92, 139–140
Adalia bipunctata (two-spotted lady beetle) 130
Aeschynomene virginica (northern jointvetch) 253
Africa 46, 64, 141, 165–166, 185, 209–210, 329
Ageratina riparia (Hamakua pamakani) 256
Ageratum conyzoides 334
Agrilus hyperici 231
Agrobacterium radiobacter 272–273, 284
Agrobacterium tumefaciens 272–273, 284
agrocin 272
Agrypus flaveolatus 40
alfalfa 139, 183
 strip harvesting 90, 91
alfalfa caterpillar (*Colias eurytheme*) 183
alfalfa weevil (*Hypera postica*) 93
Alien, movie 145
Allee effects 117
alternate hosts 94–95
amber disease 189
ambush predators 127
ambushers, entomopathogenic nematodes 173
American black cherry (*Prunus serotina*) 254
American chestnut (*Castanea dentata*) 260, 278
American elm (*Ulmus americana*) 260
Ampelomyces quisqualis 284, 289
Amsinckia intermedia (fiddleneck) 224
Amyelois transitella (navel orangeworm) 147
Amylostereum areolatum 175
Amylostereum chailletii 162
Anagrus epos 95
Anaphes iole 147
Anchomenus dorsalis 136
Anguina amsinckiae (fiddleneck flower gall nematode) 224
annual bluegrass (*Poa annua*) 253
Anoplophora glabripennis (Asian longhorned beetle) 204
antagonism, of plant pathogens and plant parasitic nematodes 261–293
 antibiosis 271–273
 induction of resistance 273–275
 parasitism 269–270, 286
 resource competition 267–269
Anthonomus grandis (boll weevil) 82, 209
antibiosis
 plant resistance strategy 326
 strategy used by antagonists 271–273
Anticarsia gemmatilis (velvetbean caterpillar) 63, 93, 197–198
antixenosis 326
ant lions (Myrmeleontidae) 127
ants (Formicidae)
 predatory 25, 31, 32, 34, 84, 89, 96, 127, 137, 160
 tending homopterans 137, 139, 161, 235
Aonidiella aurantii (California red scale) 6, 103, 104, 114–115, 116, 117
Aphelinidae 154, 167
Aphelinus jucundus 154
aphid flies (Chamaemyiidae) 134
aphid parasitoids 148, 152, 154, 156, 167
aphid predators 128, 129–131, 132–133, 136, 142
aphid virus vectors 274
Aphidencirtus aphidivorus 156
Aphididae (aphids) 6, 7, 8, 15, 63, 76, 94, 95, 130, 135, 137, 139–140, 143, 148, 154, 156, 161, 180, 190, 209, 308
Aphidiidae 52, 152, 167
Aphidius colemani 167
Aphidius matricariae 167
Aphidoletes aphidimyza 142
aphids (Aphididae) 6, 7, 8, 15, 63, 76, 94, 95, 129, 130, 135, 137, 139–140, 143, 148, 154, 156, 161, 180, 190, 209, 308
Aphis gossypii (cotton aphid) 81, 82–83, 86, 139, 141
Aphis nerii (oleander aphid) 127, 128
Aphis pomi (apple aphid) 8
Aphis spiraeicola (spirea aphid) 8
Aphytis chrysomphali 103, 104
Aphytis lingnanensis 103–105
Aphytis melinus 103–105, 114–117, 167
Apis mellifera (honeybee) 298, 311
Apoanagyrus lopezi 165–166
apples 8, 91, 96, 272, 334
apple aphid (*Aphis pomi*) 8
apple maggot (*Rhagoletis pomonella*) 8
Araneae (spiders) 33, 87, 91, 96, 127, 137–138
Araneidae (orb weaving spiders) 137
Argentina 175
Argentine ant (*Linepithema humile*) 115
Arizona 330
Arkansas 82
Armillaria mellea 265
armored scales (Diaspididae) 127
army ants 127
armyworm (*Spodoptera* sp.) 153, 178, 209
Arthrobotrys anchonia 270
artichoke 178
artichoke plume moth 178
artificial diet 72
Asclepias (milkweed) 225
Ascomycota (sac fungi) 206, 280
ash trees 330
ash whitefly (*Siphoninus phillyreae*) 40, 330
Asian gypsy moth (*Lymantria dispar*, Asian strain) 25

- Asian longhorned beetle (*Anoplophora glabripennis*) 204
- Askew, R. R. 157
- Atlantic Canada 228
- augmentation 65–67
 application 73, 74, 76
 definition 62, 325
 industry 68, 78
 macroorganisms 69–74, 142, 167
 microorganisms 74, 251–254, 282
 products 69–77, 78, 79, 142, 167, 253, 283, 284
 regulations 77, 283–284
 use for control 142, 167, 251–254, 285–286, 331–333
- autocidal control 325, 326–327
- Autographa gamma* (Silver-y moth) 155
- Australia 17, 41–43, 45, 60, 89, 98, 124, 175, 199, 201, 222, 223, 228, 230, 232, 247, 256, 302, 311, 322–331
- Bacillaceae 181, 281
- Bacillus*, antagonist of plant pathogens 283, 284, 334
- Bacillus popilliae* 188–189
- Bacillus sphaericus* 182, 188
- Bacillus subtilis* 275, 284
- Bacillus thuringiensis* 20, 25, 63, 67, 76, 79, 181–182, 188, 335
 application 76, 186
 corn 186
 cotton 186
 genetic engineering 20, 186–187, 326
 history 183–184
 mass-production 75, 185
 resistance 187, 326
 safety 185, 299–300
 use for control 182, 185–186, 331, 333
- Bacillus thuringiensis israelensis* 179, 184, 185
- Bacillus thuringiensis kurstaki* 182, 183
- Bacillus thuringiensis morrisoni* 184
- Bacillus thuringiensis “tenebrionis”* 184
- bacteria
 antagonists of plant pathogens 272, 280–281
 insect pathogenic 180, 182, 183, 188
 infection 180–181
 mass-production 185, 189
 use for control 181–189
 symbiotic 171–173, 179, 180
- Baculoviridae 191
- bait 213
- balance of nature 21
- balanced mortality hypothesis 157
- banker plants 67, 168
- bark beetles (Scolytidae) 108, 109
- Basidiomycota 253, 255, 268, 269, 280
- Bassi, Agostino 26
- beans 274
- Beauveria bassiana* 26, 208, 209
- Beauveria brongniartii* 204, 209, 211
- Beddingia siricidicola* 175–177
- beetle banks 86, 87–88, 334
- beetles (Coleoptera) 209, 234
 larval (grubs) 177, 178, 182
 predatory 142
- berries 178
- Bessa remota* 47–48, 301
- big-eyed bugs (*Geocoris*) 91, 126
- billbugs 178
- BioChon 253–254
- bioherbicide 63
- biological control
 definition 19, 20–21, 325
 history 25–27
 of arthropod pests 27–28
 of plant pathogens 29–30
 of weeds 29
 of invertebrate pests 101, 203
 of plant pathogens 259
 of vertebrate pests 199–202
 of weeds 215
 publications 27, 35
 research methodology 30–35
 cages 31–32, 108–109, 122
 direct observation 33
 evidence of presence or activity 34
 prey enrichment 33
 removal techniques 32–33
 sampling 31
 strategies for use 37
 systems for use 22–23
- biologically based pest management
 definition 20, 21
- Biomal 253
- biopesticides 63, 177, 181, 251, 282, 329, 331
- bioprotectants 65, 282
- biorational chemical agents 325, 327–328
- biorational pest control *see*
 biologically based pest management
- biotypes 52, 58, 71
- birds 13, 88, 194–198, 242
- black crust of rubber 281
- black flies (Simuliidae) 184, 185
- black tern (*Childonias niger*) 242
- black vine weevil (*Otiorhynchus sulcatus*) 209, 333
- blackberry (*Rubus* spp.) 248, 256
- blackberry leafhopper (*Dikrella californica*) 95
- Blattodea (cockroach) 209, 211
- Blepharipa pratensis* 121
- blind releases 67, 89, 168, 333
- Blossey, B. B. 243, 244
- Boettner, G. H. 303
- boll weevil (*Anthonomus grandis*) 82, 209
- bollworm (*Helicoverpa zea*) 148, 159, 320, 335
- Bombyx mori* (silkworm) 26, 298
- Bordeaux mix 4
- botanicals 327
- Botrytis* 284, 288
- Botrytis cinerea* 264, 289
- Braconidae 146, 147, 148
- Brazil 17, 29, 141, 197, 198, 208, 246, 274, 281
- Briggs, C. J. 114
- British Columbia 25, 228
- brown planthopper (*Nilaparvata lugens*) 335, 336
- Bruchidae 32
- Bt (*B. thuringiensis*) *see* *Bacillus thuringiensis*
- Buchnera* 180
- Bufo marinus* (cane toad) 124, 302
- bugs, predatory 131–132, 142
- bumblebees, greenhouse pollinators 68, 78
- Bupalus piniarius* (pine looper moth) 159
- Buprestidae 231, 236
- Burdon, J. J. 218
- Burkholderia* 283, 293
- Burma 25
- Butler, L. 299
- butterflies 238
- cabbage 92, 93, 187
- cabbage looper (*Trichoplusia ni*) 150
- cacao 96
- Cactoblastis cactorum* 222–223, 232, 308
- calicivirus 201

- California 41–43, 45, 52–53, 78, 90, 91, 94, 103, 115, 143, 216, 226, 230, 321
- California red scale (*Aonidiella aurantii*) 6, 103, 104, 114–115, 116, 117
- Calosoma frigidum* 129
- Calosoma sycophanta* 136
- Campbell Soup 335
- Camperico 253
- Canada 7, 66, 195, 228, 253
- cane toad (*Bufo marinus*) 124, 302
- Candida oleophila* 280, 284
- Carabidae (ground beetles) 87, 127, 129, 135, 136, 137, 140
- Carabus auronitens* 136
- Carcinops pumilo* 142
- cardenolides 127
- cardiac glycosides 225
- Cardiochiles nigriceps* 159
- Carduus nutans* (musk thistle) 221, 256
- Caribbean 124
- Carson, Rachel 12–14
- cassava 33, 46, 141, 197
- cassava green mite (*Mononychellus tanajoae*) 60, 141
- cassava mealybug (*Phenacoccus manihoti*) 33, 56, 60, 165–166
- Castanea dentata* (American chestnut) 260, 278
- caterpillars 93, 127, 155, 158, 177, 178, 182, 191, 193, 194, 196, 222, 223, 227, 234, 235, 240, 299, 333, 335
- Cathartona catoxantha* 47
- Cecidomyiidae (midge), predaceous 134, 142
- cecropia moth (*Hyalophora cecropia*) 303
- Centaurea* spp. (knapweeds) 229
- Centaurea diffusa* (diffuse knapweed) 220, 229
- Centaurea maculosa* (spotted knapweed) 229
- Central America 89
- Cerambycidae (long-horned beetles) 209, 211, 238
- Ceratitis capitata* (Mediterranean fruit fly) 316
- cereal aphids 31
- cereal cyst nematode (*Heterodera avenae*) 265, 292
- cereal leaf beetle (*Oulema melanopus*) 83
- cereals 86–88, 334
- Chamaemyiidae (aphid flies) 134
- Chalcididae 147
- Chalcidoidea 146, 149, 154, 156, 161, 323
- checkered beetle (Cleridae) 109
- Cheilomenes lunata* 128
- chemical pesticides 5, 6, 7, 10, 320, 323, 328
- chestnut 278, 279
- chestnut blight 260, 278–279
- Chilodnius niger* (black tern) 242
- Chile 256
- China 72, 79, 84, 86, 89, 103, 141, 166, 201, 208, 284, 334
- Chloris gayana* (Rhodes grass) 89
- Chondrilla juncea* (rush skeletonweed) 256, 257
- Chondrostereum purpureum* 253, 254
- Choristoneura fumiferana* (spruce budworm) 7, 185
- Chromaphis juglandicola* (walnut aphid) 51, 52–53
- Chromista 206
- antagonists of plant pathogens 269, 275, 280, 287
- pathogens of weeds 253
- chrysanthemums 328
- Chrysocharis gemma* 161
- Chrysolina quadrigemina* (Klamath beetle) 230, 231
- Chrysomelidae (leaf beetles) 185, 226, 227, 230, 234, 240, 241, 243
- Chrysopa rufilabris* 142
- Chrysoperla carnea* 132
- Chrysopidae (green lacewings) 6, 7, 72, 73, 91, 92, 95, 96, 132–133, 142
- Chytridiomycetes 206
- ciliates 263
- cinnabar moth (*Tyria jacobaeae*) 223, 226–227, 228
- Cirsium* 303, 304
- Cirsium canescens* (Platte thistle) 305
- Cirsium undulatum* (wavyleaf thistle) 305
- Cistothorus palustris* (marsh wren) 242
- citrus 6, 15–16, 25, 41–43, 84, 89, 103, 172, 178–179, 248, 252, 253, 334
- citrus black scale (*Saissetia oleae*) 58
- citrus red mite (*Panonychus citri*) 89, 334
- citrus root weevil (*Diaprepes abbreviatus*) 172, 178–179
- Citrus sinensis* (pera sweet orange) 274
- Citrus tristeza* 274
- classical biological control
- definition 39, 325
- economics 46, 56, 226, 230, 248
- evaluation 56–61
- examples 40, 110, 115, 256
- foreign exploration 58
- methods 56–57, 61
- natural enemy attributes 102–105
- new associations 46–47
- quarantine 58–59
- releases 59–60, 234
- success rate 44, 45, 48–51, 54, 55
- taxonomy, importance 57–58
- terminology 49
- use 43–46, 206, 255, 315, 330, 331
- Cleridae 109
- Coccinella novemnotata* (nine-spotted lady beetle) 308
- Coccinella septempunctata* (seven-spotted lady beetle) 129, 139, 140, 141, 308
- Coccinellidae (lady beetles) 63, 83, 91, 92, 127, 128, 129–131, 140, 141, 142, 248, 308
- Cochineal insect (*Dactylopius coccus*) 29, 236
- Cochliomyia hominivorax* (screwworm fly) 327
- cockroach (Blattodea) 209, 211
- cock's foot grass (*Dactylis glomerata*) 87, 88
- coconut moth (*Levuana iridescens*) 47–48, 301
- coconut palm 47–48, 195
- codling moth (*Cydia pomonella*) 8, 209
- Coelomomyces stegomyiae* 206
- coevolution 200, 314
- coffee 57
- coffee berry borer 209
- coffee leafminer (*Leucoptera coffeella*) 57
- Coleoptera (Beetles) 178, 182, 209, 234
- Coleotichus blackburniae* (Koa bug) 306, 307
- Colias eurytheme* (alfalfa caterpillar) 183
- collards 85, 121, 139
- Collego 253

- Colletotrichum gloeosporioides* 253
Colletotrichum gloeosporioides f.sp. *aeschynomene* 253
 Colombia 141
 Colorado potato beetle (*Leptinotarsa decemlineata*) 4, 16, 131, 185, 209, 333
 community ecology 120–122
 companion plants 94
 competition, in biological control of plant pathogens 267–269
 competitive displacement 103
Compsilura concinnata 111–121, 302, 303
Coniothyrium minitans 284, 334
Conotrachelus nenuphar (plum curculio) 8, 14
 conservation biological control
 antagonists of plant pathogens 289
 conservation plus enhancement 80–81
 cover crops 89
 crop management 90
 crop residue management 89–90
 definition 80, 81, 325
 enhancing natural enemies 83–96
 physical environment 93–94
 plant characteristics 90, 92
 polyculture 85–89
 providing food 95
 providing shelter 96
 reducing pesticides 81–83
 refuges 86–89
 soil 92–93, 290–292
 use for control 334
 vegetational diversity theory 85–86
 constitutive defense, plants 326
 convergent lady beetle (*Hippodamia convergens*) 72, 142, 143
 copepod 206
Copidosoma truncatellum 155
Copidosomopsis tanytmenus 154–155
 corn 70, 89, 166
 corn leafhopper (*Dalbulus maidis*) 89
Corticium sp. 269
 cosmetic damage 15–16
Costelytra zealandica (grass grubs) 181, 189
Cotesia melanoscelus 121
 cotton 86, 90, 95, 96, 139, 159, 166, 248, 275
 cotton aphid (*Aphis gossypii*) 81, 82–83, 86, 139, 141
 cotton boll weevil (*Anthonomus grandis*) 82, 209
 cottony cushion scale (*Icerya purchasi*) 39, 41–43
 cover crops 329
 crab spiders (Thomisidae) 137
 cranberries 178
 cranberry bogs 177, 333
 cranberry girdler 178
 crickets 160, 178
 cross protection 273–274
 crown gall 30, 272, 284
 crucifers 237
 cruisers, entomopathogenic nematodes 173
 crustacean 206
Cryphonectria parasitica 278–279
Cryptochaetum iceryae 43
Cryptolaemus montrouzieri 142
Ctenopharyngodon idella (grass carp) 249
 cucumber 90, 274
Culex 188
Culex pipiens quinquefasciatus 174
 Culicidae (mosquitoes) 125, 174, 179, 182, 184, 187, 188, 191, 206, 209, 333
 cultivation, crop 325
 cultural control 4, 325
 Curculionidae (weevils) 178, 220, 234–235, 238, 240, 242, 243, 247, 248
 cutworm 178
Cydia pomonella (codling moth) 8, 14, 209
Cylindrobasidium laeve 253
Cylindrosporium concentricum 281
Cyperus esculentus (yellow nutsedge) 236, 254, 255
 cyst nematode 265, 293
Cyrtobagous salviniae 246, 247
Cyzenis albicans 40

Dactylis glomerata (cock's foot grass) 87, 88
 Dactylopiidae 235
Dactylopius 236, 248
Dactylopius ceylonicus 29, 240
Dactylopius coccus (cochineal insect) 29, 236
 dairy, fly control 323–324
Dalbulus maidis (corn leafhopper) 89
 damage
 invertebrate pests, due to 97–98
 plant pathogens, due to 259
 vertebrate pests, due to 98–99
 weeds, due to 215–216
 damping off 284, 285, 286
 Darwin, Charles 19, 21
 dates 25
 DDT 4, 6, 7, 9, 14, 26, 320, 323
 deBach, P. 21
 delta-endotoxin 181
Dendroctonus frontalis (southern pine beetle) 108–109, 122
Dendrolimus spp. (pine caterpillars) 208, 209
 Denmark 14, 332, 333
 Denno, R. F. 139
 density dependence 46, 107–108, 110, 115, 293
 density independence 108, 110, 115
Dermolepida albobirtum (Greyback cane beetle) 302
 desert locust (*Schistocerca gregaria*) 209
 Deuteromycetes 206, 210, 253, 280
 DeVine 252, 253
Diachasmimorpha tryoni 316
Dialectica scariella 311
 diamondback moth (*Plutella xylostella*) 92, 187, 209
Diaprepes abbreviatus (citrus root weevil) 172, 178–179
 Diaspididae (armored scales) 127
Diatraea saccharalis (sugarcane borer) 208
Dicyma pulvinata 281
 Dietrick, E. J. 90, 91
 diffuse knapweed (*Centaurea diffusa*) 220, 229
Dikrella californica (blackberry leafhopper) 95
 Diptera (flies) 178, 209, 234
 disease, definition 180
 Dixon, A. F. G. 297
Diuraphis noxia (Russian wheat aphid) 306
 domatia 91, 92
 downy mildew 289
 Dr. BioSedge 254
 Duetting, P. S. 92
 Dutch elm disease 260

 earwigs 96
 East Asia 56

- eastern subterranean termite (*Reticulitermes flavipes*) 325
- Echium plantagineum* (Paterson's curse, Salvation Jane) 311
- ecological host range 313
- ecology, macroorganismal vs. microorganismal 262–263
- economic injury level 23–24, 110, 319, 320
- economic threshold 23, 24, 320
- economics
- beetle banks 88
 - classical biological control 46, 56, 226, 230, 248
- ecotype 58
- ectoparasitoids 152, 153
- Edwardsiana prunicola* (prune leafhopper) 95
- egg parasitoids 72, 121, 150, 167, see *Trichogramma*
- Eichhornia crassipes* (waterhyacinth) 239, 240, 330
- electrophoresis 34
- Elkinton, J. S. 303
- encapsulation 163
- Encarsia formosa* 66–67, 73, 74, 90, 167–168, 333
- Encarsia inaron* 40
- Encyrtidae 156
- endoparasitoids 152
- enemies hypothesis 85
- enemy release hypothesis 26, 39, 243
- England see United Kingdom
- Entomophaga maimaiga* 40, 120, 205, 206–208, 312–313
- Entomophthorales 205, 206, 207, 318
- Entyloma ageratinae* 256
- Environmental Protection Agency, U.S. 13–14, 252, 268
- Eotetranychus sexmaculatus* 113
- Ephestia kuehniella* (Mediterranean flour moth) 154, 155, 183
- Epidinocarsis lopezi* 166
- Epilachna varivestis* (Mexican bean beetle) 65
- epizootic 82, 93, 118, 119, 189, 190, 195, 205, 208
- epizootiology
- cycles of infection 119, 120
- equilibrium, population 110–111
- non-equilibrium theory 114
- eradication 25
- Eretmocerus eremicus* 167
- erosion 199
- Erwinia amylovora* 284, 289
- Erynnis ello* 197
- Erythroneura elegantula* (grape leafhopper) 94
- Erythroneura* spp. (grape leafhoppers) 142
- Eucalyptus* 89, 185
- Eucelatoria armigera* 148
- Euglandina rosea* 305
- Eulophidae 153, 161
- Euphorbia* 94
- Euphorbia esula* (leafy spurge) 220
- Euplectrus* sp. 153
- Europe 66, 78, 131, 136, 167, 254, 332
- European apple sawfly (*Hoplocampa testudinea*) 8
- European cockchafer 209
- European corn borer (*Ostrinia nubilalis*) 70–71, 159, 166, 183, 208, 209
- European rabbit (*Oryctolagus cuniculus*) 199–201
- European red mite (*Panonychus ulmi*) 8
- European spruce sawfly (*Gilpinia hercyniae*) 7, 195
- Eutreta xanthochaeta* 316
- Everglades 331
- fairly flies (Mymaridae) 145, 147
- fall armyworm (*Spodoptera frugiperda*) 89
- farmer training 197, 336
- fertilizer 221, 223, 245
- fiddleneck (*Amsinckia intermedia*) 224
- fiddleneck flower gall nematode (*Anguina amsinckiae*) 224
- field crops 178
- Fiji 47–48, 301
- filarial worms 185
- Finland 268
- fire ants (*Solenopsis* spp.) 161, 320
- fire blight 284, 289
- fish 13
- mosquito control 125
 - weed control 249
- flea larvae 177
- fleas (Siphonaptera), as disease vectors 200
- flies (Diptera) 178, 209, 234
- maggots (larvae) 142, 177, 178, 182
 - phytophagous 229
 - predatory 133–134, 135, 142
- Florida 175, 178, 308, 331
- flowerhead weevil (*Rhinocyllus conicus*) 303–305, 315
- flowers, antagonists protecting 284
- foil (*B. thuringiensis*) 186
- Food & Agriculture Organization (United Nations) 14, 316, 335
- food webs 120–122, 306
- foliage, antagonists protecting 284
- Follett, P. A. 307
- forestry 185
- Formicidae (ants)
- predatory 25, 31, 32, 34, 84, 89, 96, 127, 137, 160
 - tending homopterans 137, 139, 161, 235
- formulation 76, 177, 185, 208, 210
- France 52, 66, 290
- Frankliniella occidentalis* (western flower thrips) 134
- frass 158
- Frenchi cane beetle (*Lepidiotia frenchi*) 302
- Frost, Robert 279
- fruit flies (Tephritidae) 229, 238, 316
- functional response 105–106
- fungi
- antagonists of plant pathogens 268, 269, 271, 280, 289
 - arthropod pathogens 64, 190, 203, 204, 205
 - dispersal 204
 - diversity 205–206
 - ecology 92, 204–205
 - infection 203–204
 - mass production 205, 206, 208
 - use for pest control 82, 209, 211
 - nematode-trapping 270, 292, 293
 - pathogens attacking weeds 63, 254, 255, 256
 - symbiotic 162, 175, 176, 275
- Fungi Imperfecti 206, 210, 253, 280
- fungicides 5, 287
- fungus gnats (Sciaridae) 63, 134, 142
- furanocoumarins 225
- Fusarium*
- non-pathogenic antagonist 290–291, 334
 - plant pathogenic 287, 334
- Fusarium oxysporum* var. *melonis* 290
- Gaeumannomyces graminis* var. *tritici* 291
- Galerucella californiensis* 241, 243
- Galerucella pusilla* 241, 243

- Galleria mellonella* (wax moth) 33
 gall-forming fly 316
 gall-wasps 234, 243
 galls, plant 217, 224, 229, 243
Gambusia affinis (Mosquitofish) 125
 Gastropoda 178
 Gause, G. F. 263
 geminivirus 335
 genetically modified crops 5
Geocoris (big eyed bugs) 91, 126
Geocoris pallens 126
 geranium 287
 Germany 187, 194, 201
 giant African snail (*Achatina fulica*) 305
Gilpinia hercyniae (European spruce sawfly) 7, 195
Gladiolus 283, 334
Gladiolus virens 270
 glucosides 237
Glyptapanteles liparidis 316
 golf courses 253
 granulosis virus (GV) 192
 grape leafhopper (*Erythroneura elegantula*) 94
 grape leafhoppers (*Erythroneura* spp.) 142
 grapefruit trees 116
 grapes 91
 grass 188
 grass carp (*Ctenopharyngodon idella*) 249
 grass grubs (*Costelytra zealandica*) 181, 189
 grasshoppers 64, 209–211, 213, 234
 Great Britain *see* United Kingdom
 green fluorescent protein 266
 green lacewings (Chrysopidae) 7, 91, 132, 142
 Green Muscle 211
 green peach aphid (*Myzus persicae*) 318
 green rice leafhopper (*Nephotettix cinctipes*) 33
 greenbug (*Schizaphis graminum*) 148, 152
 greenhouses 65, 66–67, 74, 76–77, 78, 79, 89, 90, 91, 93, 132, 133–134, 135, 140–141, 167–168, 177, 332, 333
 greenhouse whitefly (*Trialeurodes vaporariorum*) 73, 90, 91, 167–168
 gregarious parasitism 153
 greyback cane beetle (*Dermolepida albobirtum*) 302
 ground beetles (Carabidae) 87, 127, 129, 135, 136, 137, 140
 Gryllotalpidae (Mole crickets) 175, 178
Gryllus integer 160
 guilds 217
 gypsy moth (*Lymantria dispar*) 31, 34, 40, 120–121, 122–123, 129, 136, 185, 205, 206–208, 299, 302–303, 312, 316, 327, 333
 Hagen, K. S. 95
 Halloween lady beetle (*Harmonia axyridis*) 15
 Hakataki 253
Hakea sericea (Silky needlebush) 253
Hamakua pamakani (*Ageratina riparia*) 256
 Harman, G. E. 286
Harmonia axyridis (halloween lady beetle) 15
Harpalus pennsylvanicus 139, 140
Harpobittacus nigriceps 228
 Harris, P. 221, 229
 harvested fruits and vegetables, antagonists protecting 284
 Hawaii 45, 55, 94, 256, 274, 306–307, 315, 316
 hawk moth 197
 Hawkins, B. A. 301
Helicoverpa zea (bollworm, tomato fruitworm) 148, 159, 320, 335
Heliothis virescens (tobacco budworm) 159
 Hemiptera (True bugs) 131–132, 142, 209, 234
 herbicides 5
Herpestes javanicus (small Indian mongoose) 124
Heterobasidion annosum 29, 267
Heterodera avenae (cereal cyst nematode) 265, 292
Heteropan dolens 301
 Heterorhabditidae 171–174, 177–178
Heterorhabditis bacteriophora 172, 178
Heterorhabditis indica 178
Heterorhabditis megidis 178
Hieroglyphus daganensis 64
Hippodamia convergens (convergent lady beetle) 72, 142, 143
Hirsutella rhossiliensis 292
 Hister beetles 142
 Holling, C. S. 105
 Homer 4
 honeybee (*Apis mellifera*) 298, 311
 honeydew 91, 137, 139, 156, 159, 161, 167, 235
Hoplocampa testudinea (European apple sawfly) 8
 horntails (Siricidae) 162
 host feeding 156, 166, 168
 host plant specificity, in natural enemies 236–237
 host resistance 325–326
 host specificity 46, 138
 effect on mass production 68–69
 host range 300–301
 interpreting test results 252
 predictability/potential for change 313–315
 relation to control 115
 testing host specificity 309–311
 housefly (*Musca domestica*) 323, 324
 housefly parasitoids 167
 hoverflies (Syrphidae) 94, 134, 135
 Howarth, F. G. 28, 315
 Hoy, M. A. 71
 Huffaker, C. B. 111
 Hunt-Joshi, T. R. 244
Hyalophora cecropia (cecropia moth) 303
Hylobius transversovittatus 242
 Hymenoptera 234
Hypera postica (alfalfa weevil) 93
 Hypericin 230
Hypericum perforatum (Klamath weed, St. John's wort) 230–231
 hyperparasitism
 of parasitoids 156
 of plant pathogens 269, 281
Hypoaspis miles 134, 142
 hypovirulence 278
Icerya purchasi (cottony cushion scale) 39, 41–43
 Ichneumonidae 146, 162
 Ichneumonoidea 146, 167
 idiobionts 152
 immune response, insect
 to nematodes 173
 to parasitoids 163
 immunoassays 34
 India 17, 29, 90, 103, 124
 Indonesia 335, 336

- induced defense response, plants 326
 induced systemic resistance (ISR) 275
 infective juvenile, nematode 170
 inoculative biological control
 definition 63–65, 325
 re-establishment 196
 seasonal 65
 secondary cycling 64
 use for control 63–65, 142, 167, 254, 282
 insect growth regulators (IGRs) 327
 insectary, wild 94
 insecticidal check method 32, 33
 insecticides 5, 336
 insidious flower bug (*Orius insidiosus*) 73, 92, 93, 142
 integrated pest management (IPM)
 28, 319–322, 323–336
 biointensive 322, 323
 definition 319, 325
 natural enemies and IPM 334–335
 uses 318–320, 322, 336
 interior plantscapes 74
 International Organization for Biological Control (IOBC) 27, 72
 intraguild predation 138
 inundative biological control
 application 73, 74, 76
 availability of natural enemies 78
 definition 62–63, 325
 product development 69
 use for control 62–63, 142, 167, 208, 251–253, 254, 282, 331–333
 invasive species 10–11, 215–216, 241, 330
 invertebrates, pests 97–98, 101–102
 Iran 52
 iron-limited soils 269
 Isoptera (termites) 209
 Israel 184
 Italy 278
Ixobrychus exilis (least bittern) 242

 Japan 206–208, 253, 316
 Japanese beetle (*Popillia japonica*) 13, 28, 172, 188
 jumping spiders (Salticidae) 127, 138

 Kentucky 94
Kieferia lycopersicella (tomato pinworm) 335

 Klamath beetle (*Chrysolina quadrigemina*) 230, 231
 Klamath weed (*Hypericum perforatum*) 230–231
 knapweeds (*Centaurea* spp.) 229
 Koa bug (*Coleotichus blackburniae*) 306, 307
 Koa trees 306
 Koebele, Albert 41
 koinobionts 152
 Krieg, A. 184

 lacebug (Tingidae) 314
 lacewing, green (Chrysopidae) 6, 7, 72, 73, 91, 92, 95, 96, 132–133, 142
 lacy phacelia (*Phacelia tanacetifolia*) 94, 95
 lady beetles (Coccinellidae) 63, 83, 91, 92, 127, 128, 129–131, 140, 141, 142, 248, 308
 lady bird fantasy 28
Lagenidium giganteum 206, 209
Lantana camara 219, 239, 245, 314
 larch sawfly (*Pristiphora erichsonii*) 115
 late blight, potato 259, 318
 leaf beetles (Chrysomelidae) 185, 226, 227, 230, 234, 240, 241, 243
 leaf miners 161
 leafhoppers 142
 leafy spurge (*Euphorbia esula*) 220
 least bittern (*Ixobrychus exilis*) 242
 lemon 115, 116
Lepidiota frenchi (Frenchi cane beetle) 302
 Lepidoptera 209, 234, 238
 larval 178, 182
Leptinotarsa decemlineata (Colorado potato beetle) 4, 16, 131, 185, 209, 333
 lettuce 94
Leucoptera coffeella (coffee leafminer) 57
Levuana iridescens (coconut moth) 47–48, 301
 life tables 30
Linepithema humile (Argentine ant) 115
 Linnaeus, Carl 26
 livestock 230, 327
Lixophaga sphenophori 94
Lobularia maritima (Sweet alyssum) 94, 95
Locusta migratoria 210
 locusts 63, 209, 210, 329

 longhorned beetles (Cerambycidae) 209, 211, 238
Longitarsus aeneus 311
Longitarsus echii 311
Longitarsus jacobaeae 226, 227
 Losey, J. E. 139
 Louisiana 239, 240
 Luckmann, W. H. 15
 Lycosidae (wolf spiders) 137
 Lygus bug (*Lygus hesperus*) 90, 147
Lygus hesperus (Lygus bug) 90, 147
Lymantria dispar (gypsy moth) 25, 31, 40, 120–121, 122–123, 129, 136, 185, 205, 206–208, 299, 302–303, 312, 316, 327, 333
Lymantria monacha (nun moth) 194
 Lymantriidae (tussock moths) 127, 147
Lythrum salicaria (purple loosestrife) 241–243

Macrolophus caliginosus 131
 macroorganisms for augmentation
 mass production 71–72
 natural enemy strain/species 71
 products 78–79
 release 73
 storage and transport 72–73
 Madagascar 57
Mahanarva posticata (sugarcane spittlebug) 208, 209
 Malaysia 96
 mallows (*Malva* spp.) 253
Malva spp. (mallows) 253
 Manitoba 115
 Mantodea (praying mantids) 127, 133, 134, 136, 142
 Marino, P. C. 301
 marker genes 266
 marsh 216
 marsh wren (*Cistothorus palustris*) 242
 Marshall, D. R. 218
 mass-production
 bacteria 185, 189
 fungal, invertebrate pathogens 205, 206
 nematodes, invertebrate parasites 177–178
 quality control 72, 176
 mating disruption 327, 335
 Mauritius 124
 McEvoy, P. B. 228
 McFadyen, R. E. C. 55
 mealybugs 129, 137, 142

- meat industry 201
mechanical control 322–325
Mecoptera (scorpionflies) 228
Mediterranean 103
Mediterranean flour moth (*Ephesia kuehniella*) 154, 155, 183
Mediterranean fruit fly (*Ceratitis capitata*) 316
Megarhyssa atrata 162
Megarhyssa nortoni 162
Melaleuca quinquenervia (Paperbark tea tree) 331
Meligethes aeneus (Rape pollen beetle) 85, 93
melon 290
Mermithidae 174
mesquite trees (*Prosopis* spp.) 55
Mesoseiulus longipes 142
metalaxyl 287
metamorphosis 126
metapopulation 111–113
Metarhizium anisopliae 208, 209, 211
Metarhizium anisopliae var. *acridum* 64, 209–211, 329
Metcalf, R. L. 15
methyl bromide 10
Mexican bean beetle (*Epilachna varivestis*) 65
Mexico 89, 166, 335
microbial control 63
microbial pesticides 63
microorganisms for augmentation
 formulation 76
 mass-production 75–76
 microbial strain/species 74–75
 products 79
 release 76
 storage and transport 76
microsporidia 211–213
 life cycle 211–212
 mass production 213
microtype eggs 149
midge, predaceous (Cecidomyiidae) 134, 142
mildew 284
milkweed (*Asclepias*) 225
milkweed vine (*Morrenia odorata*) 63, 252, 253
milky disease 188
mites (Acarina) 129
 biological control of weeds 234
 pestiferous 32, 83
 phytophagous 66, 89, 111
 predatory 7, 73, 78, 89, 95, 96, 111, 113, 131, 132–133, 134, 142, 333, 334
modeling
 climate 58
 fungi in soil 293
 host feeding 166
 insect pathogens 94
 population 30, 105, 107, 111–120, 121
mole crickets (Gryllotalpidae) 175, 178
molecular techniques
 for identification 53, 59
 plant pathology 265, 266
mongoose, small Indian (*Herpestes javanicus*) 124
monoculture 26
Mononychellus tanajoae (cassava green mite) 60, 141
Monterey pines 175
Moorea 305
Morrenia odorata (stranglervine, milkweed vine) 63, 252, 253
mosquitoes (Culicidae) 125, 174, 179, 182, 184, 187, 188, 191, 206, 209, 333
 as disease vectors 200
mosquitofish (*Gambusia affinis*) 125
moths 238
multiple parasitism 153
Murdoch, W. W. 114
Musca domestica (housefly) 323, 324
Muscidifurax raptor 167, 323, 324
Muscidifurax raptorellus 167
Muscidifurax zaraptor 167
mushrooms 178
 antagonists protecting 284
musk thistle (*Carduus nutans*) 221, 256
mutants 266
mycoherbicide 63
mycoparasitism 269, 286
Mycorrhizae 275, 292
Myers, J. H. 220, 229
Mymaridae (fairy flies) 145, 147
mynah bird (*Acridotheres tristis*) 124
Myrmeleontidae (Ant lions) 127
Myrothecium verrucaria 20
myxoma virus 195–200
myxomatosis 195–200, 201, 314
Myzus persicae (green peach aphid) 318
Nasonia vitripennis 167
“nasty hosts”, of parasitoids 161
natural control 19, 21–22, 108–109, 122
natural enemies
 attributes, successful 102–105, 236
 definitions 19, 102
 diversity 102
 natural selection 8, 200
navel orangeworm (*Amyelois transitella*) 147
nectar 89, 94, 156
nematodes
 entomopathogenic 63, 170, 172, 174, 176, 333
 ecological requirements 173
 formulation and application 177
 host location 173
 host specificity 173
 mass production 176
 use for biological control 175–179
 plant parasitic
 biological control of 292
 control using fungal antagonists 75
 control using fungal compounds 20
 use for biological control of weeds 224, 234
Nematophthora gynophila 265
Neochetina bruchi 240
Neochetina eichhorniae 240
Neodiprion swaini (Swaine jack pine sawfly) 157
Neoseiulus californicus 142
Neoseiulus cucumeris 63, 66, 142
Neoseiulus fallacis 142
Neozygites fresenii 82
Nephotettix cinctipes (green rice leafhopper) 33
Netherlands 14, 66, 168, 330, 331
new associations 46–47, 300
New Brunswick 7
New Mexico 330
New York State 40, 70
New Zealand 25, 55, 98, 181, 189
Nezara viridula (southern green stink bug) 306
Nicaragua 15, 89
niche 264
niche market 332
Nicholson-Bailey model 107, 111
Nicotiana attenuata 126–127
nicotine 161, 328–330

- Nilaparvata lugens* (brown planthopper) 335, 336
 nine-spotted lady beetle (*Coccinella novemnotata*) 308
Niphograptus albiguttalis 240
 nitrogen 221, 223, 245, 248
Nomadacris septemfasciata (red locusts) 124
Nomuraea rileyi 93
 non-equilibrium theory 114
 non-occluded virus 195
 northern jointvetch (*Aeschynomene virginica*) 253
 Norway 268
Nosema locustae 213
 Nova Scotia 40, 115
 nuclear polyhedrosis virus (NPV) 76, 192–193, 194–195, 196, 198
 numerical response 105, 106
 nun moth (*Lymantria monacha*) 194
 nurseries 177

 oak (*Quercus*) 122–123, 220
 oak galls 157
 oak leaf miners 157
 occlusion body 192
 Oceania 56
Oecophylla smaragdina 25, 84
 oil palm 195
 oleander aphids (*Aphis nerii*) 127, 128
 olive scale (*Parlatoria oleae*) 115
 onchocerciasis, control of 185
 onion 287
 onion basal rot 287
Ooencyrtus kuvanae 121
 Oomycetes 206, 280
Operophtera brumata (winter moth) 40, 115
Opuntia spp. (prickly pear cactus) 222–223, 232, 236, 239, 248, 308
Opuntia corallicola (semaphore cactus) 308
Opuntia vulgaris (prickly pear) 29
 oranges 111, 113
 orb weaving spiders (Araneidae) 137
 orchard trees 134, 211
 Oregon 226–228
Orgyia thyellina (white-spotted tussock moth) 25
Orius insidiosus (insidious flower bug) 73, 92, 93, 142
Ormia ochracea 160
 ornamentals 66, 73–78, 178, 333
 Orthoptera 64, 160, 178, 209–211, 213, 234
Orthotydeus caudatus 92
Oryctes rhinoceros (rhinoceros beetle) 56, 195–196
Oryctolagus cuniculus (European rabbit) 199–201
Ostrinia nubilalis (European corn borer) 70–71, 159, 183, 208, 209
Otiorynchus sulcatus (black vine weevil) 209, 333
Oulema melanopus (cereal leaf beetle) 83
 outbreak pest populations 24–25, 206

Paecilomyces fumosoroseus 209
Paecilomyces lilacinus 292, 293
Paenibacillus popilliae 182, 188–189
 painted apple moth (*Teia anartoides*) 25
 Pakistan 103
Pandora neoaphidis 92
Panonychus citri (citrus red mite) 89, 334
Panonychus ulmi (European red mite) 8
 papaya ringspot 274
 paperbark tea tree (*Melaleuca quinquenervia*) 331
 Papua New Guinea 246
Parasetigena silvestris 121
 parasitic beetles 149
 parasitic flies 148–149
 parasitic wasps 91, 146–148
 parasitoids 145
 communities 157
 definition 145
 dispersal 151
 diversity 146
 host acceptance 150–151, 160
 host defense 160–161
 host finding 158
 host habitat location 157–160
 life history strategies 149–152, 157
 oviposition 146–149
 parasitoid offense 154–155, 161, 164
 use for biological control 164–167, 208
Parlatoria oleae (olive scale) 115
 parsnip 225
Partula 305
Pasteuria penetrans 265, 292
 pasture spittlebugs 209
 pastures 93, 181, 199, 226
 Paterson's curse (*Echium plantagineum*) 311
 pathogen
 definition 180
 ecology 118
 invertebrate, basics 190
 pea 326
 pea aphid (*Acyrtosiphon pisum*) 92, 139–140
 pears 91
Pediobius foveolatus 65
 Pemberton, R. W. 314
Penicillium 264
 Pentatomidae (stink bug) 129, 131, 307
 peppers 66
 pepper weevil 209
 Pera sweet orange (*Citrus sinensis*) 274
Perillus bioculatus 131
Peromyscus leucopus (White-footed mouse) 34, 122, 123
 pest control
 cosmetic damage 15–16
 history 4–5
 need for alternatives to chemical pesticides 10–13
 use of pesticides 5, 328
 pests 3, 15–16, 23–24
 diversity 22–23
 invasive species 10–11, 16–17
 monocultures 16
 native species 16
 outbreaks 24–25
 pesticide resistance 7–9
 resurgence, target pest 5, 6
 secondary pests 6, 8, 14, 81, 133
Pesticide Conspiracy, The 322
 pesticide treadmill 5–9, 81, 322
 pesticides, synthetic chemical
 anti-pesticide activism 322–331
 availability 10
 health and environmental effects 13–15
 history 4, 9–10
 market 5
 misuse 13–14, 15, 322
 regulation 10, 13–15
 relation to other types of control 325

- resistance 7–9
 selective 83
 use 328
Phacelia tanacetifolia (lacy phacelia) 94, 95
Phaenopsitylenchidae 175
Phanerotoma flavitestacea 147, 155
Phasmarhabditis hermaphrodita 178, 179
Phenacoccus manihoti (cassava mealybug) 33, 56, 60, 165–166
 pheromones 108, 320, 327, 335
Phlebiopsis gigantea 267–268, 280, 282
 Phoridae 161
Photorhabdus 171–173
Phragmidium violaceum 256
Phycomyces blakeleeanus 269
Phyllachora huberi 281
Phyllonorycter testudinea (spotted tentiform leafminer) 8
 physical control 322–325
 physiological host range 312, 317
 phytoalexins 326
Phytoecia coerulescens 311
Phytophthora spp. 178, 285
Phytophthora cinnamomi 275
Phytophthora infestans 259, 318
Phytophthora palmivora 252, 253
 Phytoseiidae 91, 134
Phytoseiulus persimilis 66, 78, 142, 332
 pied-billed grebe (*Podilymbus podiceps*) 242
 pine 108–109, 122, 157, 166, 175–177, 267, 275
 pine caterpillars (*Dendrolimus* spp.) 208, 209
 pine looper moth (*Bupalus piniarius*) 159
 pine wood wasp (*Urocercus gigas*) 162
 pisatin 326
 plant disease, biological control of
 foliage diseases 288–289
 post-harvest diseases 288, 289
 seed and root diseases 285–286, 287
 stem and crown diseases 288
 plant ecology, relevant to biological
 control of weeds 224–229
 plant defenses 225
 plant growth promoting
 rhizobacteria (PGPR) 275
 plant parasitic nematodes, biological
 control of 292
 plant pathogens and plant parasitic
 nematodes
 antagonists of 261–262, 269
 antagonist activity 261–262, 266
 discovering antagonists 277–279
 diversity 279
 mass production, of antagonists 265, 268, 275, 283, 292
 protective use 265, 285
 using antagonists for control 281–293
 losses due to 259
 plant pathogens, used for biological
 control of weeds 251–257
 plant stress hypothesis 221, 226–227, 228
 Platte thistle (*Cirsium canescens*) 305
 plums 91
 plum curculio (*Conotrachelus nenuphar*) 8
Plutella xylostella (diamondback moth) 92, 187
Poa annua (annual bluegrass) 253
Podilymbus podiceps (pied-billed grebe) 242
Podisus maculiventris 129
Poecilostictus cothurnatus 159
 pollen 89, 94, 95, 132–133, 134, 156, 334
 polyculture 85–89
 polydnaviruses 163, 164
 polyembryony 153
 pomegranate 40
Popillia japonica (Japanese beetle) 13, 28, 172, 188
 population theory
 Allee effects 117
 delayed density dependence 107, 108
 density dependence 107–108, 110, 115, 293
 density independence 108, 110
 density vague relations 107
 equilibrium, population 110–111
 metapopulations 111–113
 modeling 105, 111–120, 121
 non-equilibrium theory 114, 115
 pathogen reservoir 119
 refuges 113–117
 regulation 106–107, 114, 115
 potatoes 259, 318–320, 322
 Potter, M. C. 26
 powdery mildew 91, 274, 284, 289
 Poxviridae 192, 199
 praying mantids (Mantodea) 127, 135, 136, 138, 142
 precocious parasitoid 154–155
 predators 124–143
 behavior of, invertebrates 105–106, 126–127
 host specificity 138
 ingestion by, invertebrates 128
 intraguild interactions 138–139
 invertebrate 126, 129
 prey defense (invertebrates) 127–128, 135
 use in biological control 140, 142
 vertebrate 124–125, 302
 Price, P. W. 157
 prickly pear cactus
 Opuntia spp. 222–223, 232, 236, 248, 308
 Opuntia vulgaris 29
 primary parasitism 156
Pristiphora erichsonii (larch sawfly) 115
 Proctotrupoidea 146
 proovigenic parasitoids 156
Prosopis spp. (Mesquite trees) 55
 prune leafhopper (*Edwardsiana prunicola*) 95
 Prune trees 95
Prunus serotina (American black cherry) 254
Pseudacteon 161
 Pseudomonadaceae 280
Pseudomonas 283, 290, 291
Pseudomonas fluorescens 186, 280
Pseudomonas syringae 284
Pseudomonas tolaasi 284
Pseudomyza flocculosa 289
 Pteromalidae 167
Puccinia canaliculata 254, 255
Puccinia carduorum 256
Puccinia chondrillina 256, 257
 purple loosestrife (*Lythrum salicaria*) 241–243
 Pyralidae 222, 235, 240
 pyrethroids 328
Pyrilla perpusilla (sugarcane leafhopper) 90
 pyrrolizidine alkaloids 311
Pythium 265, 285, 287, 334
Pythium acanthicum 269
Pythium oligandrum 280, 284
 quarantine 58–59
Quaylea whittieri 58

- Quebec 157
Quercus 122–123, 220
- r*-*K* continuum 218, 226–227, 228, 264–265
- rabbit calicivirus disease 201
- rabbits 98, 199, 201, 314
- radiata pines 175
- rangeland 202, 210, 213, 222, 230, 304
- rape pollen beetle (*Meligethes aeneus*) 85, 93
- rare and endangered species 317
- Raven (*B. thuringiensis*) 186
- red-headed cockchafer 209
- red imported fire ant (*Solenopsis invicta*) 139
- red locust (*Nomadacris septemfasciata*) 124
- refugia, from Bt-engineered plants 187
- Reoviridae 192
- resistance
 to *Bacillus thuringiensis* 187, 326
 to pesticides 7–9, 142, 185, 323
 to viral pathogen 200
- resource concentration hypothesis 85
- resurgence, target pest 5
- Reticulotermes flavipes* (eastern subterranean termite) 325
- Reunion 57
- reverse-order method, testing host specificity 309, 310
- Rhabditida 171
- Rhabdoscelis obscurus* (sugarcane weevil) 94
- Rhagoletis pomonella* (apple maggot) 8
- rhinoceros beetle (*Oryctes rhinoceros*) 56, 195–196
- Rhinocyllus conicus* (flowerhead weevil) 303–305, 315
- rhizobacteria 275, 292
- Rhizoctonia* 265, 285, 334
- Rhizoctonia solani* 286
- rhizosphere 267
- rhizosphere competence 267
- Rhodes grass (*Chloris gayana*) 89
- Rhopaea verreauxi* 188
- Rhysella persuasoria* 162
- Rhyssomatus marginatus* 220
- rice 96, 125, 166, 253, 334, 335, 336
- Riley, C. V. 41
- risk assessment 316
- Risley, C. 220
- river blindness, control of 185
- river grape (*Vitis riparia*) 92
- Roberts, W. 26
- Rodolia cardinalis* (Vedalia beetle) 39, 41–43, 56, 129
- Romanomermis culicivorax* 174, 179
- Root, R. B. 85
- root diseases 285
- root knot nematodes 270, 275, 293
- roots, antagonists protecting 284
- rootworm 89
- rotation, crop 325
- row crops 133
- rubber 197, 281
- Rubus* spp. (blackberry) 248, 256
- rush skeletonweed (*Chondrilla juncea*) 256, 257
- Russian wheat aphid (*Diuraphis noxia*) 306
- rust fungi 254, 255–256, 265, 274
- Rutz, D. A. 323
- sac fungi (Ascomycota) 206, 280
- safety, of biological control 297
 beneficials 298
 effects on endemics 298, 301–308
 indirect effects 308
 preventing non-target effects 315–317
 testing host specificity 309–311, 313
 vertebrate 298, 299–300, 302
- St. John's wort (*Hypericum perforatum*) 230–231
- Saissetia oleae* (Citrus black scale) 58
- salt cedar (*Tamarix* spp.) 216
- Salticidae (jumping spiders) 138
- Salvation Jane (*Echium plantagineum*) 311
- Salvinia molesta* 218, 246–248
- sampling 319–320
- sanitation, crop 325, 335
- Saturniidae (silk moths) 303
- sawflies 191, 234
- scale insects 129, 130, 137, 190, 234, 235–236, 248, 249
- scale parasitoids 167
- Scaphinotus* spp. 137
- scarab larvae 178, 182
- Scarabaeidae 188, 302
- Schistocerca gregaria* (desert locust) 209
- Schizaphis graminum* (greenbug) 148, 152
- Schlenger, E. I. 90, 92
- Sciaridae (fungus gnats) 63, 134, 142
- Sclerotinia* 284, 334
- Sclerotium rolfsii* 286
- Scolytidae (bark beetles) 108, 109
- scorpionfly (Mecoptera) 228
- screwworm fly (*Cochliomyia hominivorax*) 327
- Scutelleridae (shield bug) 307
- secondary infection 64
- secondary pest outbreaks 6, 7, 8, 14, 81, 133
- secondary plant compounds (or metabolites) 128, 225, 237
- seeds, antagonists protecting 284
- seed-feeding wasps 234
- semaphore cactus (*Opuntia corallicola*) 308
- Senecio jacobaea* (tansy ragwort) 223, 226–227, 228, 248
- Sepik River 246
- Serratia entomophila* 181, 182, 189
- sesame 314
- Sesbania punicea* 220, 248
- seven-spotted lady beetle (*Coccinella septempunctata*) 129, 139, 140, 141, 308
- shield bug (Scutelleridae) 307
- siderophores 269
- Silent Spring* 13
- silk moths (Saturniidae) 303
- silkworm (*Bombyx mori*) 26, 298
- silky needlebush (*Hakea sericea*) 253
- silver bullet approach, to pest control 328
- silver-y moth (*Autographa gamma*) 155
- Siphonaptera (fleas) 177, 200
- Siphoninus phillyreae* (ash whitefly) 40, 330
- Sirex noctilio* 162, 175–177
- Siricidae (horntails or wood wasps) 162, 175–177
- Skeeter Doom 179
- Slobodkin, L. 101
- slugs 178, 179
- Smith, H. S. 26, 230
- smut fungi 256
- snails 137, 178, 179
- Solenopsis invicta* (red imported fire ant) 139
- Solenopsis* spp. (fire ants) 161, 320
- solitary parasitism 153
- South Africa 17, 55, 211, 220, 243, 248, 253, 256

- South America 185
 South Pacific 195
 Southeast Asia 195, 335
 southern green stink bug (*Nezara viridula*) 306
 southern pine beetle (*Dendroctonus frontalis*) 108–109, 122
 soybeans 93, 198, 253
Spalangia cameroni 167
 species complex 59
 spider mite predators 142
 spider mites (Tetranychidae) 7, 66, 78
 spiders (Araneae) 33, 87, 91, 96, 127, 137–138
Spilochalcis sp. 147
 spirea aphid (*Aphis spiraeicola*) 8
 spittlebugs 209
Spodoptera frugiperda (fall armyworm) 89
Spodoptera sp. (armyworm) 153, 178, 209
 spotted alfalfa aphid (*Therioaphis maculata*) 321
 spotted knapweed (*Centaurea maculosa*) 229
 spotted tentiform leafminer (*Phyllonorycter testudinea*) 8
 spruce 7, 194, 195
 spruce budworm (*Choristoneura fumiferana*) 7, 185
 Sri Lanka 29
 stability, system 110–111
Steinernema carpocapsae 73, 171, 178
Steinernema feltiae 178
Steinernema glaseri 178
Steinernema riobrave 172, 178–179
Steinernema scapterisci 175, 178
 Steinernematidae 171–174, 177–178
 Steinhilber, E. A. 183–184
 Steinkraus, D. C. 82
 sterile insect release 326–327
 Stern, V. M. 320
 stink bug (Pentatomidae) 129, 131, 307
 storage
 of macroorganisms 72–73
 of microorganisms 76
 stored fruits and vegetables 284
 stranglervine (*Morrenia odorata*) 63, 252, 253
Stratiolaelaps miles 131
 strawberry 78, 332
Streptomyces 334
Streptomyces griseoviridis 280, 284
 strip harvesting 90
 Stumpout 253–254
 succession
 microbial communities 265
 parasitoid communities 157
 Sudan 330
 sugar beet 274
 sugar cane 94, 124, 166, 302
 sugarcane borer (*Diatraea saccharalis*) 208
 sugarcane leafhopper (*Pyrilla perpusilla*) 90
 sugarcane spittlebug (*Mahanarva posticata*) 208, 209
 sugarcane weevil (*Rhabdoscelis obscurus*) 94
 superparasitism 153
 suppressive soils 30, 265, 290–293
 sustainable pest control 328–330
 Swaine jack pine sawfly (*Neodiprion swainei*) 157
 Sweden 14, 268
 sweet alyssum (*Lobularia maritima*) 94, 95
 Switzerland 94, 268
 symbiotic association 162, 171–173, 175, 176
 synovigenic parasitoids 156
 Syrphidae (Hover flies) 94, 134, 135
 systemic acquired resistance (SAR) 274–275
 systems approach, to pest control 328–330

 Tachinidae 40, 47, 48, 94, 148–149, 160, 301, 302, 303, 306
 take-all decline of wheat (disease of wheat) 291
Tamarix spp. (salt cedar) 216
 tansy ragwort (*Senecio jacobaea*) 223, 226–227, 228, 248
Taphrocerus schaefferi 236
 target pest resurgence 6
 Tasmania 185
Teia anartoides (painted apple moth) 25
Teleonemia scrupulosa 314
 Tephritidae (fruit flies) 229, 238, 316
 termites (Isoptera) 209
 Tetranychidae (spider mites) 7, 66, 78
Tetranychus urticae (two-spotted spider mite) 8, 14, 78, 332
 Texas 109
Thanasimus dubius 109
Therioaphis maculata (spotted alfalfa aphid) 321
 thistle 303, 304
 Thomas, M. B. 329
 Thomisidae (crab spiders) 137
 threshold density, requiring control 23, 320
 thrips (Thysanoptera) 63, 66, 142, 209, 234
 Thysanoptera (thrips) 63, 66, 142, 209, 234
 tillage, effects on natural enemies 93
Trioxys pallidus 52
 tobacco 159, 161, 328
 tobacco budworm (*Heliothis virescens*) 159
 tolerance, plant resistance 326
 tomatoes 66, 274, 335
 tomato fruitworm (*Helicoverpa zea*) 148, 159, 320, 335
 tomato pinworm (*Kiefferia lycopersicella*) 335
 total systems approach, to pest control 328–330
 transgenic plants (*B. thuringiensis*) 186, 325, 326
 transport, of macroorganisms 73–78
 trap crops 325
 tree plantations 253
Trialeurodes vaporariorum (greenhouse whitefly) 90, 91, 167–168
Trichapion lativentre 220, 248
Trichilogaster acaciaelongifoliae 243
Trichoderma 269, 283, 286–288, 334
Trichoderma hamatum 286
Trichoderma harzianum 284, 286, 287
Trichogramma 28, 74, 78–79, 149–151, 166–167, 335
Trichogramma brassicae 167
Trichogramma minutum 167
Trichogramma nubilale 159
Trichogramma ostrinae 70–71, 167
Trichogramma pretiosum 167
 Trichogrammatidae 167
Trichoplusia ni (cabbage looper) 150
Trichopoda pilipes 306
 trichomes 90, 92
 Trinidad 124
Trioxys pallidus 51, 52–53
Trissolcus basalus 306
 trophic interactions 122
 triungulin 149

- true bugs (Hemiptera) 131–132, 142, 209, 234
- turf 177, 178, 253
- tussock moth (Lymantriidae) 127, 147
- two-spotted lady beetle (*Adalia bipunctata*) 130
- two-spotted spider mite (*Tetranychus urticae*) 8, 14, 78, 332
- Tydeidae 91, 92
- Typhlocyba pomaria* (white apple leafhopper) 8
- Typhlodromalus aripo* 141
- Typhlodromus occidentalis* 113
- Typhlodromus pyri* 134
- Tyria jacobaeae* (cinnabar moth) 223, 226–227, 228
- Uganda 314
- Ulmus americana* (American elm) 260
- ultra-low volume application 208, 210
- United Kingdom 17, 66, 87, 94, 157, 168, 179, 268, 334
- Urocus gigas* 162
- Uromycladium tepperianum* 256
- Urophora affinis* 229
- Urophora quadrfasciata* 229
- Uruguay 175
- USSR 166
- van den Bosch, R. 52, 321–322
- van Leeuwenhoek, A. 26
- van Lenteren, J. 49
- Vedalia beetle (*Rodolia cardinalis*) 39, 41–43, 56, 129
- Vedalia cardinalis* 42
- vegetables 166, 335
- greenhouse 66–67, 68, 78, 167, 333
- velvetbean caterpillar (*Anticarsia gemmatalis*) 63, 93, 197–198
- vertebrates
- biological control agents 124–125, 249
- pests 98–99, 199–201
- Verticillium* 292
- Verticillium chlamydosporium* 75, 265, 266, 270, 293
- Verticillium dahliae* 275
- Verticillium lecanii* 76, 93, 209, 333
- vineyards 94, 142
- viral pathogens, infecting animals 190
- virulence
- changes in 195–200
- pathogen 200
- viruses
- general biology 191
- invertebrate pathogens 56, 63, 93, 190, 191–194
- dispersal 193
- environmental persistence 192
- genetic engineering 198
- infection 192–193
- mass production 196–197, 198
- use for control 194–197, 199
- vertebrate pathogens 195–200, 202
- Vitis riparia* (river grape) 92
- von Tubeuf, C. F. 26
- Wagner, D. L. 299
- walnut 52–53
- walnut aphid (*Chromaphis juglandicola*) 51, 52–53
- Washington 291
- waterhyacinth (*Eichhornia crassipes*) 239, 240, 330
- wavyleaf thistle (*Cirsium undulatum*) 305
- wax, on leaf surface 92, 93
- wax moth (*Galleria mellonella*) 33
- Weaver, Sigourney 145
- webworm 178
- weeds, biological control of 215
- diversity of feeding damage 219–224
- diversity of natural enemies 217–218
- evaluation of biological control 231
- invertebrate natural enemies 233–236
- microbial natural enemies 251–257
- natural enemy attributes 51
- natural enemies, locating hosts 236–237
- natural enemy host specificity 237–238
- natural enemy host specificity testing 238
- use of invertebrates for control 234, 238–248
- use of microbes for control 251–254
- use of vertebrates for control 248, 249
- weeds, problems due to 215–216
- weed strips 94
- weevils (Curculionidae) 178, 220, 234–235, 238, 240, 242, 243, 246, 247, 248
- root feeding 178
- western flower thrips (*Frankliniella occidentalis*) 134
- wetlands 241, 331
- wheat 86, 87, 94, 291, 334
- white apple leafhopper (*Typhlocyba pomaria*) 8
- white grubs 209
- whiteflies (Aleyrodidae) 66, 74, 90, 129, 131, 137, 190, 209, 335
- whitefly parasitoids 167
- white-footed mouse (*Peromyscus leucopus*) 34, 122–123
- white-spotted tussock moth (*Orgyia thyellina*) 25
- winter moth (*Operophtera brumata*) 40, 115
- wolf spiders (Lycosidae) 137
- wood boring beetles 178
- wood wasps (Xiphydriidae and Siricidae) 162, 175
- woody stems and roots, antagonists protecting 284
- wool industry 201
- World Health Organization 15
- Wright, M. G. 70
- Xanthomonas campestris* pv. *poae* 253
- Xenorhabdus* 171–173
- Xiphydriidae (wood wasps) 162, 175
- yeast, antagonist of a plant pathogen 280, 288, 289
- yellow nutsedge (*Cyperus esculentus*) 236, 254, 255
- Yemen 25
- Zoophthora phytonomi* 93